

Magnetic field assisted fluidization – a unified approach.

Part 8. Mass transfer: magnetically assisted bioprocesses

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Abstract

Part 8 of the series *Magnetic field assisted fluidization (MFAF)* – *A unified approach* deals with biochemical upstream (production) and downstream (separation) processes performed in magnetically assisted reactors. Basic reactor principles well-implemented in chemical engineering science are used to analyze the existing situation of those special types of mass transfer devices. The hydrodynamics of all systems reviewed in the previous parts of the series (G-S, L-S and G-L-S) serves as a foundation assuring proper understanding of their mass transfer performances. Thorough analysis of magnetically assisted bioprocesses is performed together with suitable devices for their performance. Part 8 refers not only to reactors with magnetic biosupports but also to processes with free-suspended cells imposed to magnetic field action and micro devices for upstream processing.

Keywords: downstream processes; free-cell magnetically assisted processes; magnetically assisted reactors; magnetic field assisted fluidization; upstream processes.

Preface

Part 8 of the ongoing review series that deals with bioreactors should be considered as a natural continuation of Part 7 (Mass transfer). However, unlike Part 7, the present analysis does not refer to flow patterns in reactors from the traditional chemical engineer's viewpoint. This review directly focuses attention on the process performance, but when it is necessary to explain the mass transfer results the hydrodynamic background will be referred to. This does not mean omitting a topic of relevance but only a method that will use the basis

of chemical reactors to explain the effect in their biotechnological counterparts. Taking into account the general difference between the processes in chemical and biochemical reactors, it is maintained that from a hydrodynamic viewpoint, flow organization, methods that affect mixing, separation or mixing the phases all have a common background. Approaches resembling a chemist's viewpoint that have been published in some articles that explain everything with the presence of "magic magnetic fields" are definitively avoided. The last comment simply means that magnetically assisted bioreactors cannot be considered as strange devices utilizing the "magic magnetic power", as it appears in some of the studies analyzed in this review, but as well-designed equipment and processes staying on the shoulders of the fundamentals in physics, chemistry and biology.

Several compressive reviews have been published on the problems of magnetic biocarriers (Safarik et al. 1995), on magnetic separation techniques in microbiology (Safarik and Safarikova 1997, 1999a,b), on process engineering of enzyme reactors (Yeon et al. 2009), on separation in environmental processes (Larsson and Mosbach 1979, Anastasakis 1999), on ethanol production (Liu CZ et al. 2009), etc. The present review does not repeat their contents but will refer to these reference sources. This review follows the style of the previous parts of the series and could be considered as a continuation of the concept developed in "Magnetic field assisted bioreactors" (Hristov and Ivanova 1999).

A note about the reference style: this review series uses the Harvard system of references by (Name, Year). It was successfully applied to all previous parts without any problems because when huge information should be analyzed it was more convenient than the Vancouver system with numbers in parentheses. However, with Part 8 the author has met a problem with citations of astonishing numbers of articles published by Chinese authors with the same surnames. Because of this, in some cases the references are cited as the surname with the first author's initials:

- Example 1: Li ZY et al. (2007) and Li Y et al. (2007a,b) are different articles by different authors. Without the initials, all of them would be Li et al. (2007).
- Example 2: The same problem exists, for example, with Zhou LM et al. (2010) and Zhou ZD et al. (2010), thus following the rule references should be cited as Zhou ZD et al. (2010a,b) which is incorrect because the authors are different.

This approach should be accepted as an attempt to avoid a specific problem rather than as a rule.

1. Introduction

1.1. Briefs on the topics analyzed

Fluidized bed reactors have been successfully employed to carry out two-phase (liquid-solid) and multiphase (gas-liquid-solid) reactions in the chemical and biochemical industries. Parts of the problems concerning their application have been solved by development of ferromagnetic particle liquid

fluidization and bed stabilization by an external magnetic field (Siegel 1988). Magnetic field assisted (MFA) bioreactors (Rosensweig 1979, Liu YA et al. 1991, Estevez et al. 1995) containing fluidized magnetizable beads are attractive devices for bioprocessing (Burns and Graves 1987, Webb et al. 1996), both for upstream and downstream processes. The creation of such bioreactors requires a span of knowledge covering various scientific fields: hydrodynamics, electricity and magnetism, mass transfer, etc. This attractive fluidization technique has been successfully applied for different applications in biotechnology, including:

- continuous protein recovery (Terranova and Burns 1989, Chetty and Burns 1991, O'Brien et al. 1996),
- continuous filtration of cell debris (Gellf and Boudrant 1974, Terranova and Burns 1991),
- enzyme processing (Larsson and Mosbach 1979, Burns and Graves 1987, Moffat et al. 1994, 1995),
- ethanol fermentation (Birnbau and Larsson 1982, Burns and Graves 1985, Al-Hassan et al. 1991, Ivanova et al. 1996, Liu CZ et al. 2009),
- continuous affinity chromatography (Burns and Graves 1987, Lochmuller and Wigman 1987),
- plant cell culture processing (Bramble et al. 1990).

Several reviews have been published on the problems of magnetic biocarriers (Safarik et al. 1995) and separation techniques in microbiology (Safarik and Safarikova 1997, 1999a,b), process engineering of enzyme reactors (Hailling and Dunhill 1980) and separation in environmental processes (Larsson and Mosbach 1979). The present paper differs from these overviews and tries to focus attention on the following engineering points:

- magnetic and electromagnetic field effects on cell growth and metabolism, and consequent effects on process performed;
- magnetic carriers used;
- application investigations with magnetic carriers performed by various research groups;
- what is MFAB: main concept and features applicable in biotechnology;
- analysis of the equipment-reactor design and magnetic systems;
- analysis of the operating modes in order to clarify their effect on the bioprocesses;
- an attempt to create a classification of the operating modes adequately corresponding to the bioprocessing of interests involving magnetizable solids;
- advantages and limits of magnetic field assisted bioreactor (MFAB) applications in bioreactors;
- microreactors employing microbeads;
- downstream processing involving magnetizable beads in either MFAB applications as bioreactors and bioseparators.

1.2. Review targets and outlines

This review addresses mainly biotechnological applications in two basic directions: upstream and downstream processes.

The main concept in the arrangement and the analysis of the information published so far follows the already established style in the preceding parts of this review series. The biotechnological applications are specific to some extent, because they, in contrast to the chemical processes (Part 7), work under moderate conditions, i.e., almost ambient temperatures and pressures. However, avoiding the complexity of the working conditions in chemical reactors, the biotechnological counterparts set up new challenging questions and problems addressing mainly the preparation of the magnetic biosupports, field effects on living organisms, etc. In this context, the preparation of magnetic beads is extensively discussed in this review not only as supports directly applicable in magnetically assisted bioreactors but as technical solutions offering wide applications beyond those reported in the literature. Precisely, some of the magnetic particles analyzed are not developed for magnetically assisted bioreactors, but the technology of preparation, the specific characteristics and the test experiments offer successful performance in potential magnetic field assisted fluidization (MFAF) applications.

To create a systematic review on magnetically assisted bioreactors is not an easy task because there have been no attempts in this direction for years. The last (in fact the first one ever published) review by Hristov and Ivanova (1999) is around 10 years old. Actually, it precedes the appearance of the present review series and the data collected in it require a new reading and relevant systematization, following the style of all reviews on MFAF published to date. In this context, we address those specific elements in the design of the magnetically assisted bioreactors which allow performing a comparative analysis with the chemical reactors developed on the same basis.

Beyond the point just mentioned, this review tries to collate data and performs analyses of magnetically assisted reactors which do not exactly implement magnetically assisted fluidization (MAF) but use magnetic particles controlled by external magnetic fields. In this direction, the magnetic separators for downstream and magnetic micro and nanodevices are also encompassed.

Very often, among chemists and biologists, the magnetic field is like a magic tool that might enhance a certain process with living cells or bacteria. Most of those experiments are rather naïve because they consider only external field assistance and report phenomenological data without any analyses. Moreover, as it will be commented further, the experimental conditions in such experiments are often non-reproducible because many factors, either from the hydrodynamic background of the experiments or the magnetic system design, are incompletely reported. Nevertheless, the outcome of section 3 of the present review will help the readers to create their own basis for further developments of such devices, staying on the shoulders of the work already done.

The problems under review are grouped into three major parts:

1. Classifications addressing operating modes and types of magnetically assisted bioreactors and separation processes.

2. Upstream processes referring to magnetically assisted free-cell reactors and those with magnetic beads used as biosupports, including:

- Free-cell bioreactors assisted by magnetic or electro-magnetic field (extremely low and high frequency) effect on processes not employing magnetic biosupports.
- Low-density loads bioreactors with coarse magnetic supports.
- High-density loads magnetically assisted bioreactors utilizing coarse magnetic biosupports, both anaerobic (two-phase, L-S) and aerobic (three-phase, G-L-S).
- Micro bioreactors based on new microscale technologies.

3. Downstream processing

- Separations employing magnetically assisted beds.
- Specific separations with magnetically controlled particles.
- Micro and nanodevices for downstream processes employing magnetic particles.

All previous parts of this review series (Hristov 2002, 2003a,b, 2004, 2006, 2007a, 2009) will be briefly referred in the text as Part 1, Part 2, Part 3, Part 4, Part 5, Part 6 and Part 7, respectively.

2. Classifications

Prior to reviewing the processes at issue, let us draw a simple classification which will allow easy recognition of the methods and operating modes. We address two basic directions:

1. Type of magnetically assisted reactors with regard to the phases involved in the process.
2. Type of magnetization modes and operating scenarios.

The first point appeals for clear definitions of the hydrodynamic background of the processes. In the case of chemical reactors (see Part 5, Part 6 and Part 7), the hydrodynamic studies are well developed in contrast to the biotechnological counterparts where such information is completely missing. To avoid these drawbacks existing in the literature, we will use the background established in the previous parts of this series to reconstruct the process conditions with the scarce data available, where this would be possible.

The second point particularly addresses the operating modes including a simultaneous action of fluid flow, magnetic field and granular media. This will permit explanation of the results with a platform already designed for the chemical reactor and well-developed in the previous parts of this series. This is a special task because in the bioprocess reviewed in this article such an approach is missing that hinders the comparative analysis. Hence, step-by-step, we enter a “virgin territory” which is full of data and sporadic experiments but without any systematization creating the fundament of knowledge.

2.1. Types of magnetically assisted bioprocesses

The magnetically assisted bioprocesses are classified here into two main groups:

A) Free-cell assisted processes without magnetic bodies (biosupports) in the magnetic field. The term Free-cell simply refers to the fact that the living cultures and enzymes are not immobilized on magnetic carriers. The free-cell processes can be generally divided into two subgroups with regard to magnetic field action on the culture medium:

- A1) processes with working volume entirely subjected to the magnetic field action;
- A2) processes where the magnetic field acts periodically on a small amount of the culture medium. These are reactors with recirculation where the field acts on a small tube through which the entire culture medium is recirculated.

B) Magnetically assisted processes with magnetic carriers for immobilization of living cultures and enzymes. In general, they are two-phase (L-S) or three-phase (G-L-S) systems, but organized in two principle groups depending on the amount of the magnetic carriers in the working volume, namely:

- B1) with low amount of magnetic carriers operating as free-suspended bodies in the culture medium with agitation driven either by mixer (L-S) or gas (G-L-S), or space and time varying magnetic field. Such systems form shallow beds of solids at the vessel bottom when the agitation is ceased. The term used in this review is “low-density loads” processes;
- B2) high amount of magnetic carriers forming thick packed beds at the reactor bottoms and allowing performance of MAF. The term used in this review is “high-density loads” processes.

2.2. Some important notes about the classifications

Taking into account that we have to analyze either anaerobic or aerobic processes in both groups (A and B), the straightforward way to link them to the well-established facts in MAF is to refer to them as two-phase (L-S or G-L) and three-phase (G-L-S) processes, respectively. This separation is general and accounts only the fact: Are solids carriers employed or not? The culture medium is considered as a single phase even though it consists of cells and culture liquid. In cases where a strong amount of biomass is produced, this will particularly be taken into account and commented.

2.3. Operating modes

2.3.1. Magnetization modes The operating modes are, in fact, the same as those established for MAF (Part 1), namely:

- Magnetization FIRST with a packed bed of carriers initially magnetized and then undergoing fluidization.

- Magnetization LAST with initially fluidized carriers and external field control of the magnetic phase movement.

As regards the other processes, such as those from free-cell (A1 and A2) or low-density loads systems (B1), it is difficult to create a slender generalizing scheme on the basis provided from the literature. In some specific cases, we will draw subclassifications facilitating the analysis and paving the road to more general analysis but at this moment this is a difficult task.

2.3.2. Granular flow patterns The granular flow (particles) used in the bioprocesses (upstream and downstream) can be introduced into the working volume through several operating modes, including:

- Solids batch mode concerning a limited amount loaded into the reactor and then fluidized by either magnetization FIRST or magnetization LAST mode (see Part 1 for definitions).
- Moving beds with magnetization FIRST or LAST when the particles enter the reactor from the top and exist at the bottom with a countercurrent fluid upflow.
- Solids LAST with magnetization FIRST means application of the field to the empty reactor and then the particles start to enter the working volume. This operating mode is typical of microscale reactors where the gravity forces are not enough to create a stable packed bed and to hold the particles magnetic forces are required.

Upstream processes

3. Magnetically assisted free-cell bioreactors

This section addresses some more frequently investigated processes with the only goal of showing the basic results and trends. Not all published literature is referred to. The principle approaches, commonly used technique, magnetic fields, microorganisms (yeasts and bacteria) are at issue. The principle targets include:

- Cell and bacterial proliferation and growth when exposed to magnetic fields.
- Fermentations assisted by magnetic fields in both encircled reactors or in fermentor with recirculation.
- Analysis of common results, conflicting data and interpretation of the data published.

3.1. Basic concept and overview of magnetic effects on living cultures

The quest for efficient processes in bioenergy provokes many non-conventional approaches for stimulation of the living cultures involved in the processes of producing biomass, ethanol, biodiesel (Silva et al. 2009), etc. In an effort to boost the production and manipulate the microorganisms for the most advantageous production performance, many studies have been performed by magnetic stimulation of their growth

and metabolic behavior. The information about various applications of magnetically assisted processes has not been consolidated yet as knowledge due to missing trustworthy replica experiments, unclear experimental conditions, various important but non-monitored conditions, etc. (Hunt et al. 2009).

Therefore, the basic concept of field assisted living organisms is to force or decelerate their growth and metabolic production. Some of them, such as *Saccharomyces cerevisiae* and *Spirulina platensis*, are good examples of living cultures directly related to the mass production of food and fuel (Khan and Rashmi 2010) and the attempts are to augment their performance under magnetic exposures. By contrast, *Escherichia coli* should be either killed (or decelerated) or boosted to grow and, as it will be shown in this section, strong static and low-frequency time-varying fields work in this direction rather successfully. Only static and low-frequency field actions are analyzed. Processes, mainly proliferation and growth studies, in the radio-frequency range are not at issue in this review due to two main reasons:

1. limited applications, mainly at a small laboratory scale which is impossible to enlarge towards industrial applications;
2. the fields of radio-frequency range commonly have negative effects on the living organisms, whereas we address only those applications which can provide more food, fuel and drugs, thus meeting the increasing demand of society for a better life.

Readers interested in broader aspects of magnetic and electric field effects on living microorganisms in the radio-frequency range can find some answers in the review by Hunt et al. (2009). The present review does not have a generalizing ambition in this direction, but only processes relevant to those performed in heterogeneous bioreactors with magnetic biocarriers will be discussed. This will allow elucidating some basic points, namely:

- Does the field stimulate the growth and metabolic activity when it acts alone?
- Are basic conditions created in the reactor such as the mechanical mixing of the medium, the basic macroscopic mechanisms controlling the process?
- Do the above mechanisms overlap in the experiments performed, which of them dominate and is there any room for synergetic effects?
- Does some specific feature of the experimental conditions mask the mixing effects and attribute the final result to the assisting magnetic field?

Answering these questions will allow finding the right way to:

- What in the future design of magnetically assisted bioprocess should be improved?
- What is the real role of the magnetic field in the heterogeneous reactors with magnetic biocarriers: a pure hydrodynamic one leading to reduced external diffusion mass-transfer resistances or a synergetic one with magnetic field boosted growths and metabolisms?

The magnetic field can lead to positive or negative effects on the growth and metabolism of living organisms. The most investigated area is that of near-field processes with predominately magnetic fields of various topologies. Hunt et al. (2009) provide an extensive summary and tabulated data about magnetic treatment of various microorganisms. The most important issues with regard to the bioprocesses at issue in the present review are the growth and the metabolic effects. Growth is a response of the organism to the surrounding conditions and positive or negative effects could indicate how the biosynthetic pathways can be boosted by the external magnetic field. This area of the magnetically assisted bioprocesses is discussed in detail by Hunt et al. (2009) in view of the intimate changes of the magnetized living structure. The approach in this review is a little bit different: only those processes which can be implemented in magnetically assisted reactors with magnetic biocarriers will be discussed. Several systematically performed studies will be analyzed next.

Magnetically assisted free-cell bioreactors do not use biocarriers for immobilization and simply combine traditional devices for performance of bioprocesses and external magnetic field generators. As mentioned in the classification section, these reactors fall into two subgroups:

1. continuously assisted bioprocesses with working volumes entirely surrounded by external magnetic fields,
2. processes with recirculations where a small part of the culture medium is magnetized for a given period of time and then goes back to working volume.

By contrast, the fields applied to the bioprocesses can be classified as:

- Magnetic fields (the near-field regime) predominately generated by coils with steady-state, slowly varying in time and pulsing modes.
- Electromagnetic fields with both electric and magnetic components, with ratio between 0.1 and 10, operating in the far-field regimes with typical oscillating frequency of 100 kHz or more (Hunt et al. 2009).

Taking into account the general concept of the review, only magnetic fields operating in the near-field regime will be discussed here. Initially, we will present a phenomenological overview at a glance, prior to a detailed analysis of some representative studies.

3.2. Reactors with entirely magnetically assisted working volumes

Studies in reactors entirely encircled by magnetic systems address mainly cell proliferation and growth: the reactor scale is that of a Petri dish or a shaking flask. However, the magnetic systems span a broad range from huge superconducting electromagnets to small coils energized by the common electric set. The small scale proliferation and growth studies provide essential results that are, in general, confirmed, when the fermentations are performed in laboratory scale fermentors. No industrial applications exist in this class of bioreactors, most of the studies are purely academic, focus

Table 1 Synthetic wastewater in the experiments of Yavuz and Celebi (2000, 2003).

Substrate	Concentration (g/l)
Glucose	10.0
Yeast extract (Lab M)	1.0
KH ₂ PO ₄	0.35
Na ₂ HPO ₄	1.825
(NH ₄) ₂ SO ₄	0.244
NaCl	0.015
CaCl ₂ ·2H ₂ O	0.0198
MgSO ₄ ·7H ₂ O	0.0614
FeCl ₂ ·4H ₂ O	0.0032

on fundamental problems, and to some extent, are incomplete and cannot answer the variety of questions emerging from the data published. However, without analysis of these studies, it is impossible to interpret the large scale reactors.

Two important questions are answered in this section:

1. Can the magnetic field force the production of living microorganisms?
2. Is it a magic tool or only a physical field allowing in a better way to improve the reactor performance as an addition to the environment already created in fermentors by the stirrers, blowing gas, etc.?

3.2.1. Magnetically assisted activated sludge processes

There exist only few studies that use magnetic field effects in wastewater treatment but mainly with magnetic adsorbents for removing contaminants (Ozaki et al. 1991). Yavuz and Celebi (2000, 2003a) performed detailed studies on magnetic field effects on activated sludge kinetics under batch conditions. The model wastewater used in these studies is presented in Table 1 while the activated sludge was obtained by aeration from the local wastewater treatment plant in Manisa (Turkey). The experiments performed in two similar reactors, with and without magnetic fields (Figure 1),

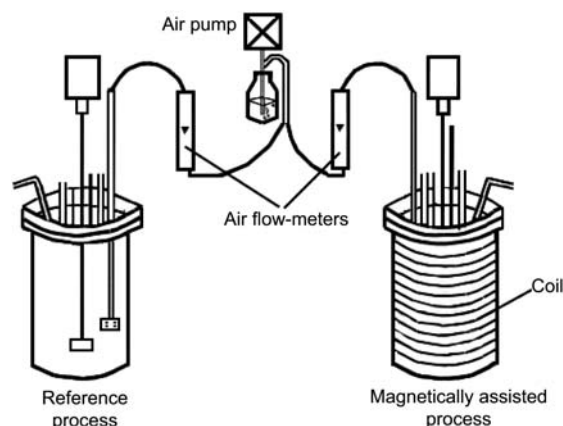


Figure 1 Experimental set-up of Yavuz and Celebi (2000, 2003a,b) – schematically. Additional data: two reactors of 300 ml capacity. Solenoid: 15 cm tall, 5 cm in diameter; 1325 turns of copper wire.

allowed comparison of the effects of the field action to those under conventional conditions. An axial field (generated but solenoid surrounding the reaction vessel) was used in DC, AC (50 Hz) and on-off modes. The choice of a certain magnetization mode was not clearly motivated but it was combined with changes in the medium pH values from 6.0 to 8.5. Each experiment lasted 40 h. The main results will be briefly outlined next.

pH effects

The variations in the medium pH keeping the field constant at 17.8 mT are illustrated in Figure 2A–C. In general, the lag time (the time where the substrate is started for removal) in the absence of a magnetic field is slightly larger ($8.5 \div 11$ h) than those in magnetized sludge ($8.0 \div 10$ h). This was considered as a positive effect of the magnetic field effect on the system start-up phase. In the context of the pH effects, Yavuz and Celebi (2000) reported that there was no correlation between the microorganisms growing rates with and without magnetic field applied. In some cases, the respective rates in magnetized sledges were lower than those in conventional (non-magnetic) processes. Only positive effect of the magnetic field applied was detected at $\text{pH} \geq 7.5$ where both the substrate removal and the microorganism growth rates were larger than those in the non-magnetic system (Figure 2C). Moreover, the maximum concentration of microorganisms in the magnetized system is higher resulting in shortened substrate disappearance time. No explanation of this synergetic effect between the optimal $\text{pH}=7.5$ and the field assistance was reported, but this reference value of pH was kept in all experiments where the field intensity was varied irrespectively of the magnetization mode used.

Field intensity effect

The field intensity was practically negligible and the large times observed were within the range of $9.0 \div 11$ h but the substrate removal increased at around 44% (based on the control non-magnetic reference process) as the field intensity was increased up to approximately 17.8 mT (Figure 2D). Further increase in the field intensity significantly reduced the substrate removal attributed to the magnetic field reducing the growth rate of the microorganisms (Yavuz and Celebi 2000). A short-time experiment at $B=46.6$ mT lasted only 0.13 h and resulted in reduction of the amount of microorganisms produced to a minimal value of 1.2 g/l; this was performed by the longest glucose disappearance time observed under such conditions.

Magnetization mode

The magnetization on fluidized beds of magnetic beads is well known (Part 1, Part 2, Part 5 and Part 7). However, in the absence of a ferromagnetic phase it can only be related to field effect related to eddy currents generation in the conducting medium (consisting of an aqueous substrate and microorganisms) by AC and on-off (pulsed field with 2 s on–2 s off) magnetizations. Yavuz and Celebi (2000) reported a slightly negative effect (with the AC 50 Hz field) in

both the substrate removal r (Figure 3A,B) and the microorganism growth rates. Similarly, no effect was detected of the pulsed magnetization (Figure 3C). It was concluded that the time-varying field reduced the positive effect of the DC field observed under similar process conditions. These results are obvious because the eddy currents induced in both the substrate and the living organisms (consisting mainly of water) cause inhibition processes in the cells (see the comments later in this section).

An effect similar to that reported by Yavuz and Celebi (2000) was described by Chen and Li (2008) in relation to polyhydroxyalkanoates (PHAs) synthesis from short-chain fatty acids: an activated sludge (1.2 MLSS/l) in a sequential batch reactor (SBR) was magnetically exposed. These authors reported that at high-field intensity of 42 mT the lowest PHAs biosynthesis rate was observed; the maximum was observed at approximately 21 mT. In general, these observations are close to those reported by Yavuz and Celebi (2000), irrespectively of the differences in the compositions of the biosystems used. Chen and Li (2008) mainly stressed the attention on the operational conditions relevant to SBR operations, whereas the field exposure (four different reactors working at fixed field intensities at 0, 7, 21 and 42 mT) effects were not well studied. In addition, the phenomenological analysis of Chen and Li (2008) provides only links to explanations provided by Blank and Soo (1998a) and Blanchard and Blackman (1994) about the field effects on the enzyme activity (no personal viewpoint was expressed), including:

- The field promotes conformation changes in certain proteins, more specifically in enzymes, by means of linked specific ions of magnesium, manganese, calcium and iron, as specific cofactors, beginning the performance of the enzymatic processes and increasing their potentiality (Blanchard and Blackman 1994).
- There are always unpaired electrons during the enzymatic reactions, where the applied magnetic field could affect the reaction by changing the electron spine states of some intermediate products.
- The field could interact with moving charges (Blank 1995) and change their velocities, that is, a classical magnetic field effect on moving charged solids. In this context, if the charge could be associated with the biological function, as in the case of an enzyme, then the function would be altered (Blank and Soo 1998a).

Process kinetics

The field effect on the macroscopic process kinetics was investigated by Yavuz and Celebi (2003a) through the Monod model:

$$\mu_M = \frac{\mu_{M-\max} S}{K_s + S}, \quad \frac{dX}{dt} = \mu_M X \quad (1)$$

under conditions equal to those commented above and reported by Yavuz and Celebi (2000).

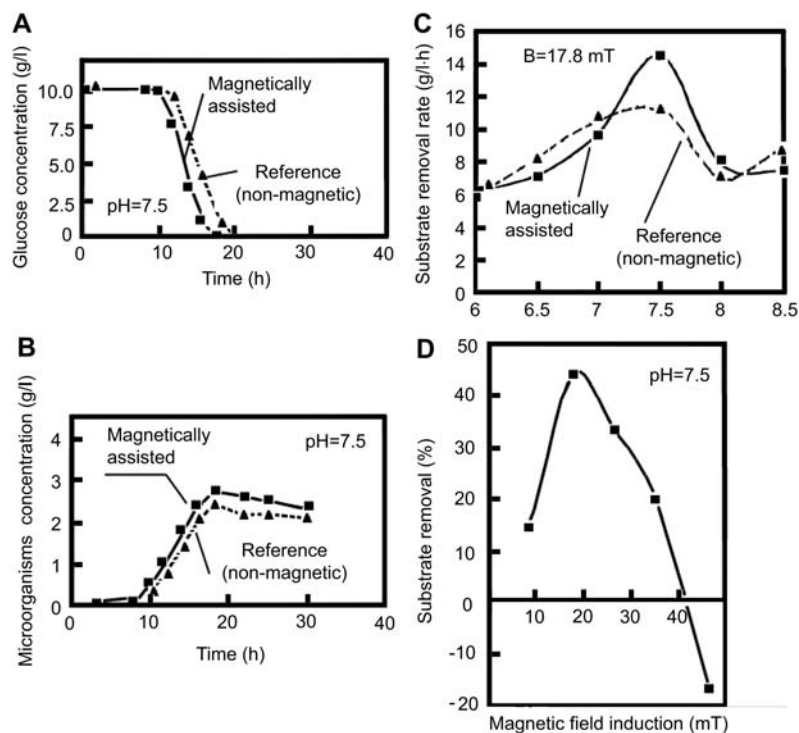


Figure 2 Time variation of the process outcomes (A and B) and effects of the process conditions on substrate removal (C and D). Adapted from Yavuz and Celebi (2000). (A) Variations of the glucose concentration in time. (B) Variations of the microorganisms concentration. (C) Effect of the substrate pH on the substrate removal rate at the optimal field intensity. (D) Magnetic field (DC) effect on the substrate removal at the optimal pH=7.5.

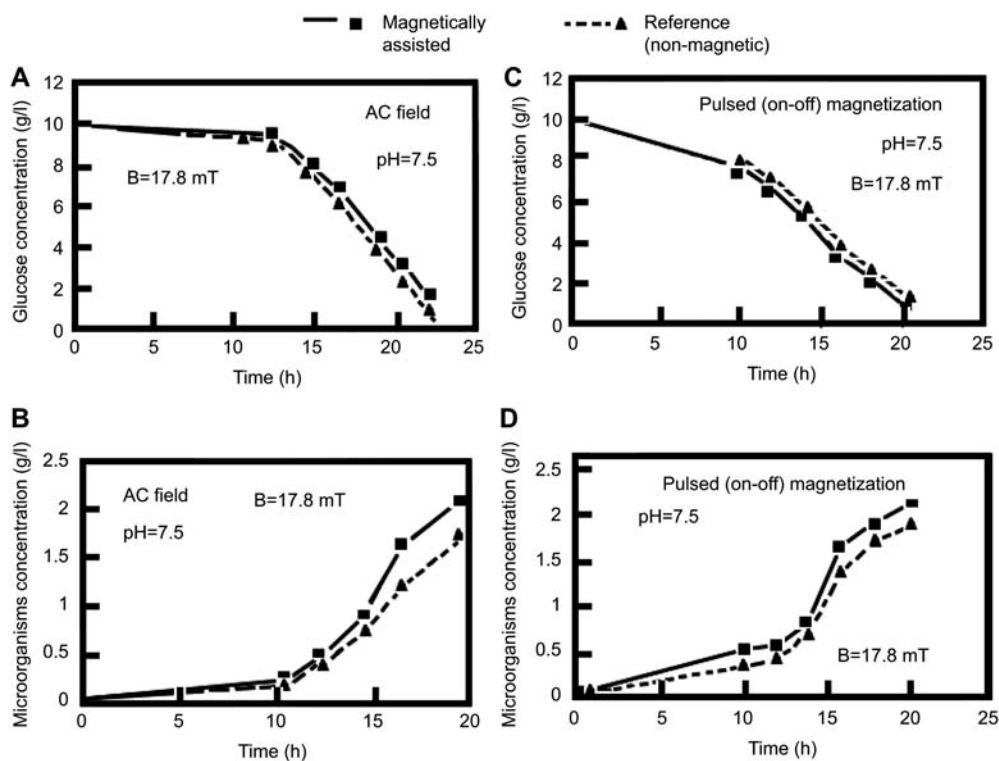


Figure 3 Magnetic field effect on the glucose consumption and the microorganism growth. Time-varying magnetization: AC (50 Hz) (A) and (B); pulsed (on-off) (C) and (D). Adapted from Yavuz and Celebi (2000).

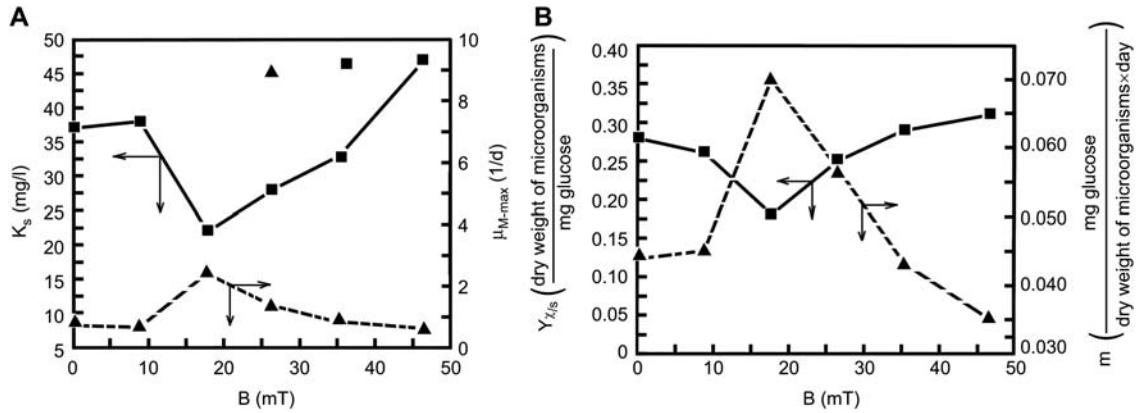


Figure 4 Magnetic field effect on the parameters of the Monod model. Graphs based on data summarized in tables 1 and 2 of the original work of Yavuz and Celebi (2003a,b).

The field effects on $\mu_{M-\max}$ and K_s are illustrated by Figure 4A,B.

The yield coefficient $Y_{x/s}$ and the maintenance factor m emerging in the equation

$$-\frac{dS/dt}{dX/dt} = \frac{1}{Y_{x/s}} + m \frac{X}{dX/dt} \quad (2)$$

are presented in Figure 4.

In general, there are no field effects at low- and high-field intensities. The best performance is presented by the parameters obtained under $B=17.8$ mT where $\mu_{M-\max}$ attains a maximum of 15.8 ± 0.5 (1/d) (Figure 5A), i.e., approximately 92.7% increase. The K_s values exhibit a minimum at $B=17.8$ mT (Figure 5B) that implies highest affinity exhibited

that leads to lowest production cost. Based on the treated data it was established that the positive field effects on the microorganism affinity were observed within the range from $B=17.8$ mT to $B=35.5$ mT. Under the same condition, the yield coefficient $Y_{x/s}$ and maintenance factor m , representing the sludge production, have little effects on the effluent quality (Figure 5C,D). Small values of $Y_{x/s}$ means high rate of substrate consumption and low sludge production. The latter simply implies a small size of the reactor and low production costs. In contrast, high values of the maintenance factor m indicate that the cells consume substrate for supporting their own lives without new cell production: this allows only small quantities of sludge to be handled. Under the best field induction of $B=17.8$ mT, the minimum of $Y_{x/s}$ was approximately 0.18 ± 0.03 (Figure 5C). At higher fields from

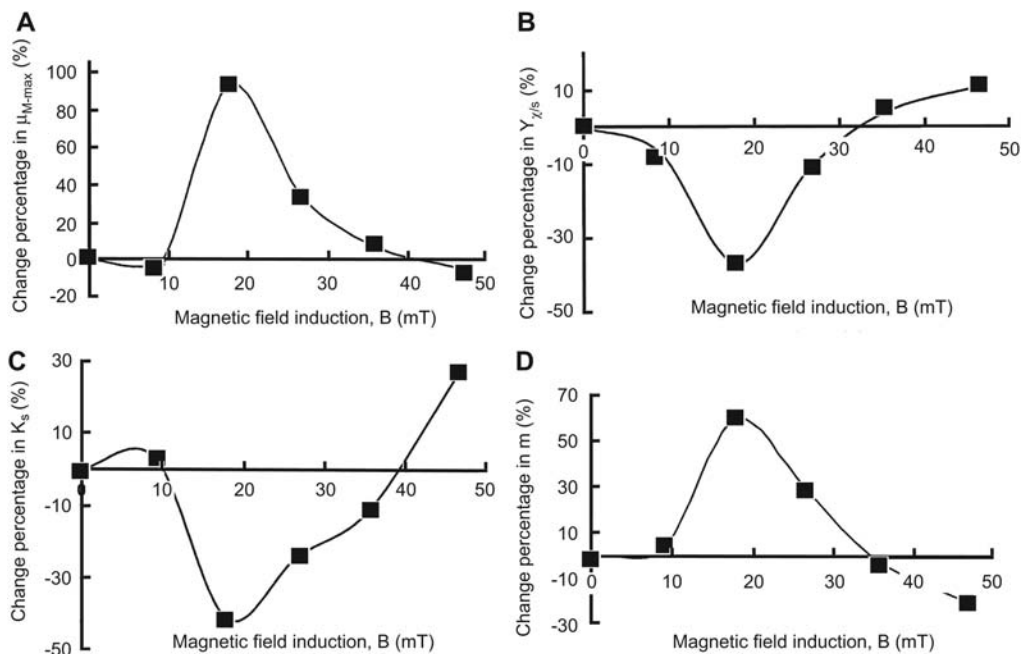


Figure 5 Variations of the parameters of the Monod models with changes in the magnetic field induction. Adapted from Yavuz and Celebi (2003a,b).

$B=35.5$ mT up to $B=42$ mT the values of 0.29 ± 0.02 were obtained. The latter simply implies that within this range the field effect is undesirable. The field effect on the maintenance factor m is illustrated in Figure 5D.

Biofilm growth on magnetic particles

The process analyzed above was part of the first large-scale study performed concerning boosting wastewater treatment in activated sludge column by magnetic fields. As a rational consequence of the field effect on the sludge activity under external field, a fluidized bed column with biofilms on magnetic particles has been developed (Yavuz and Celebi 2001). Magnetic polystyrene-based particles (average diameter of $547.5\ \mu\text{m}$) formed as compounds of polystyrene and 6 g of magnetite powder formed as pellets (pressing in forms) were used for test experiments in a fluidized bed column surrounded by two coils (Figure 6A). The use of magnetic supports avoids problems in keeping the fluidized sludge (due to the air blowing through it) in an almost homogeneous state. This is a reasonable solution, bearing in mind that in such a system the external diffusions resistance can be reduced significantly due to the high fluid solids relative velocities.

Fluidized bed tests

The biofilm growth was investigated with synthetic wastewater (the composition is not listed here) flowing through a bed arranged in a Pyrex glass column (1.5 ID and 70 cm in height). The optimal conditions of $\text{pH}=7.5$ and $B=17.8$ established in the free mixed sludge systems (Yavuz and Celebi 2000, 2003a,b) were used as a process background. The attention was focused on the time evolution of the film attached to particle surfaces and the magnetization mode. The time evolution of both attached and suspended organisms parallel to the glucose concentration are illustrated by plots in Figure 6B. The stable film thickness was attained after 25–30 h, followed by adjustment of the column operation conditions ($\text{pH}=7.5$ and $B=17.8$). An optimal film thickness of approximately $173\pm8\ \mu\text{m}$ was obtained.

The attempts to establish the optimal magnetization modes addressed experiments with a steady-state field (DC) and pulsed (on-off) magnetization. The steady-state field creates a stable structure of particles forming aggregates that consequently evolves into large clumps covered by biofilms, and, as a result, decreases the process efficiency. In this context, the on-off magnetization manifested itself as the mode assuring a better performance of the process. The results are

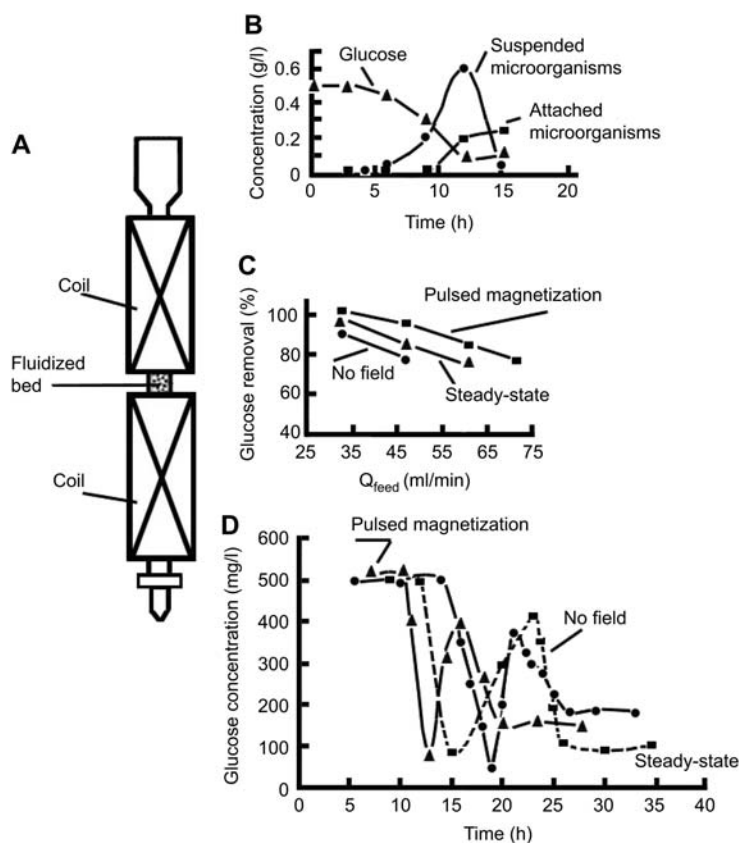


Figure 6 Biofilm growth on magnetic polystyrenes particles and magnetization mode effect on the process performance. Adapted from Yavuz and Celebi (2001, 2003b). (A) Experimental set-up, schematically (Yavuz and Celebi 2001, 2003b). (B) Changes in concentrations of substrate and microorganisms both in suspended and attached forms with time (Yavuz and Celebi 2001, 2003b). (C) Substrate removal performance at different flow rates and magnetization conditions (Yavuz and Celebi 2001, 2003b). Recirculation flow rate of 150 ml/min. (D) Effluent glucose concentration by time at different magnetization modes (Yavuz and Celebi 2003b).

reasonable, because during the off-period the bed becomes fluidized, the particles are mixed in the fluid, the aggregates are broken, and thus the formation of clumps due to magnetic forces and massive biofilm growth is avoided. At the same time, the on-period assures low-external diffusion resistance conditions for the mass transfer at the biofilm-fluid interface. Formation of clumps was observed at very low fed rates even though pulsed magnetization was applied. The conditions to the film growth under such circumstances are almost the same as those under steady-state DC magnetization, because the particle mixing is almost ceased. The simultaneous effect of the fed rate and the magnetization mode on the glucose removal is shown in Figure 6C,D.

Common problems of the magnetically assisted activated sludge processes

The common problems of magnetically assisted processes with activated sludge for wastewater treatment are briefly outlined, including:

- The processes with suspended (not attached) microorganisms are not a promising version for consequent increases in scale. The field created is not strong, but the absence of magnetic particle in the working volume leads to high losses of magnetic energy.
- Devices such as those used by Yavuz and Celebi (2000, 2003a) can be used at a laboratory scale to establish the optimal conditions for the process performance, under batch conditions of course. The increase of the reactor in size needs a fluidized bed column with magnetic biosupports for development of the biofilms and a process performance under high-feeding rates.
- The magnetization mode is a rather important factor in the adequate process development and maintenance. The steady-state field is the better option in batch systems with free suspended cells in contrast to the pulsed magnetization mode. In contrast, the column experiments with attached biofilms show that pulsed magnetization creates better conditions. There is no room for contradiction because in the batch system the steady field assistance is accompanied by an intensive stirring of the medium by a blade mixer. Hence, any formations of sludge aggregates are avoided. In column experiments, the pulsed magnetization creates conditions almost the same as those in the batch systems with mixer and DC fields. Therefore, the conditions to perform a mass transfer at the fluid-biofilm interface are almost the same. In the column, the mechanical stirrer is replaced by a high-feeding rate.
- The general problem of the scale-up cannot be solved by axial magnetic systems (used in the studies reviewed). This is an ordinary problem of all processes at issue and it will be discussed at large in this review article (see also all preceding reviews from Part 1 to Part 7).

3.2.2. Magnetically assisted *Saccharomyces cerevisiae* growth The results of magnetic field effects on *Saccharomyces cerevisiae* are somewhat controversial and with conflicting results. We will address two types of experiments:

- Experiments where proliferation response of yeast is considered only.
- Experiments with a holistic performance of fermentation processes involving *S. cerevisiae*.

3.2.2.1. Proliferation of yeast in response to magnetic field application Many articles have been published on the problem but we will comment on results of some of them where information and analyses are of primary importance for elucidation of the problem. Enough literature sources are provided by Fiedler et al. (1995), Mehedintu and Berg (1997) and Anton-Leberre et al. (2010), respectively.

Alternating magnetic fields

The experiments considered yeast proliferation response to a magnetic field in the range 10–100 Hz and amplitudes from 0.5 to 0.9 mT, generated by a Helmholtz pair (5 cm in a diameter and with uniform field over 10% around the central coil axis) (Mehedintu and Berg 1997).

Experiments that lasted over 10 h (with sampling every 2 h) revealed different yeast growth rates depending on the magnetic field amplitude. It follows that the cell number decreases in the field exposed yeasts (Figure 7A) when 0.2 mT (50 Hz) is applied and an increase of approximately $25 \pm 5\%$ when the field amplitude is 0.5 mT (Figure 7B). The scanning of the frequency range revealed two positive responses at 15 Hz and 50 Hz (Figure 7C) and inhibition at 40 Hz (Figure 7D). The two peaks in Figure 7C correspond to approximately 20% increase in the cell number for 10 h continuous exposure to the field. Furthermore, these experiments resulted in decrease in both the volume and the radius of distribution of the stimulated cells.

Some different results were found by decreasing the temperature of the medium: increase in the cell growth at 15 Hz of approximately $25 \pm 5\%$, whereas the positive effect at 50 Hz disappeared. In general, the study of Mehedintu and Berg (1997) could serve as an example of an undeveloped study even though the concept is correct and focuses on basic problems.

By contrast, Fiedler et al. (1995) used an oscillating magnetic field generated by a Helmholtz coil (via inductive coupling) to produce 0.28–12 mT magnetic field at 50 Hz. An exposure of 9 h to treat *S. cerevisiae* was performed. With conditions almost the same as those of Mehedintu and Berg (1997), these authors observed a maximum growth of 8 g/l of biomass under 0.5 mT magnetic field exposure and 6.8 g/l of biomass for the cells untreated. The positive window for cell proliferation was found at 0.5 mT. Furthermore, Fiedler et al. (1995) mentioned that at fields lower than 1 mT the cell proliferation could be enhanced. However, despite the positive results no definite explanations were developed on the basis of the experiments. Several concepts were referred, including: changes in the transmembrane voltage, Ca^{2+} distribution, enzyme turnover rate owing to local oscillation of the ion concentration (the ion activation model of Schimmelpfeng and Dertinger 1993) or influence of their radical reactions (Zhang and Berg 1992); in addition to dipole polar-

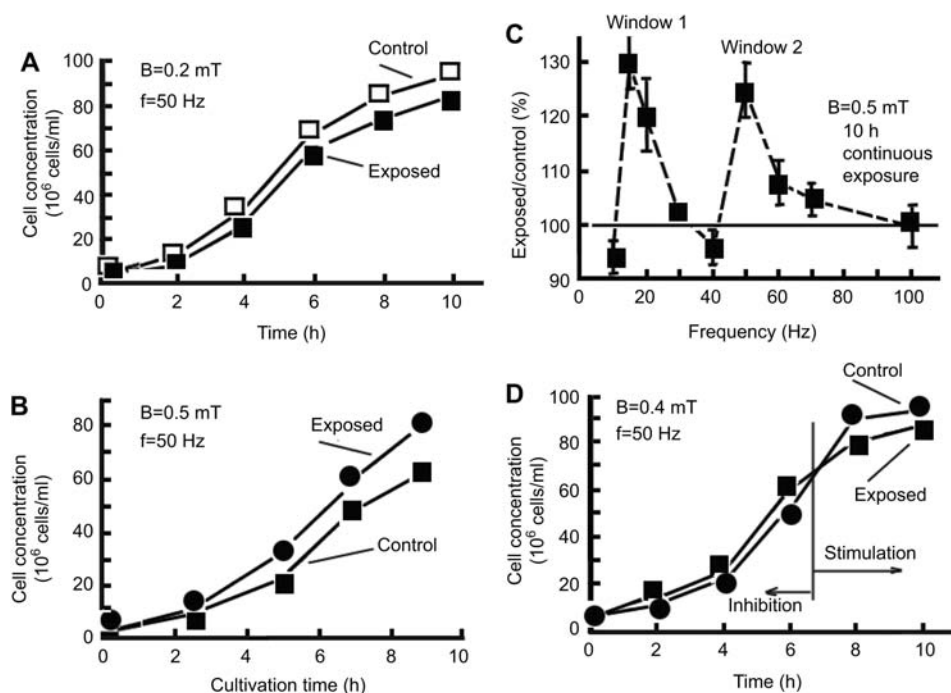


Figure 7 Experiments of Mehedintu and Berg (1997) on proliferation of *Saccharomyces cerevisiae*. (A) Inhibitory effects of low-intensity field. (B) Stimulatory effect at 0.5 T. (C) Frequency dependence of the proliferation. (D) Characteristic growth curve for inhibited proliferation. The positive effects occur after 7 h of exposure.

ization of membrane components enhanced Ca^{2+} in flux, etc. No final discrimination of the effects and mechanisms was done.

The results of Novak et al. (2007) on *S. cerevisiae* proliferation in 50 Hz (10 mT) for 24 min confirm the general tendency: decrease in the number of cells after the exposure and deceleration of the growth. The effect is similar to that reported for *Paracoccus denitrificans* (Fojt et al. 2007) and *Escherichia coli* (Strasak et al. 2002, Fojt et al. 2004) because the experimental conditions were the same (50 Hz, 10 mT), as discussed later in this section.

Static magnetic fields

Saccharomyces cerevisiae biomass growth was investigated by Muniz et al. (2007) in fields created by permanent magnets (Figure 8A) and conditions simulation of ethanolic fermentation (see later in this review). This report claims that for 24-h exposure to a field (550 mT) of 200 ml culture medium, the biomass growths in both the exposed and the control samples were almost the same during the first 8 h of fermentation. The growth of the magnetized yeast was high whereas that of the control (non-magnetized) sample was moderate. In fact, the magnetized yeast demonstrated 3.7 times superior growth rate. At the same time, the biomass value for 24-h exposure attained a level of 2.5 times greater than that of the control (Figure 8B). Moreover, the sugar depletion in the magnetized yeast culture was higher than that in the control beyond the point of 8 h to the end of the process. In this context, the depletion rate of the average

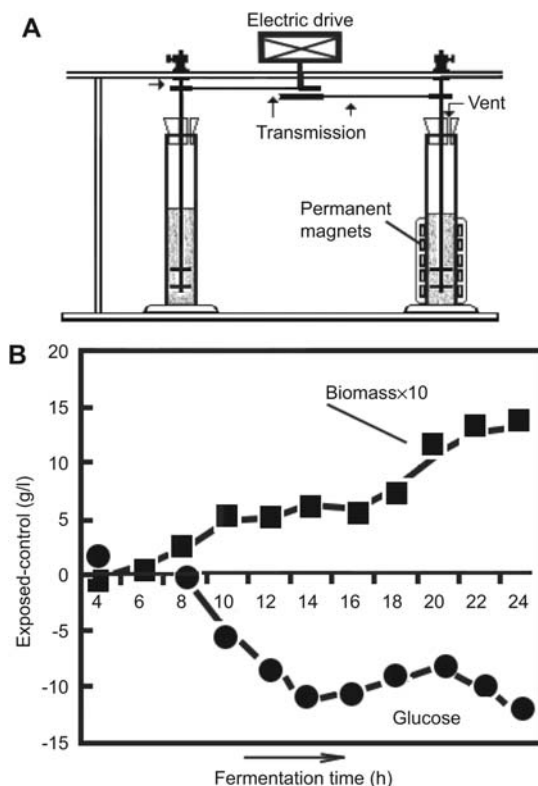


Figure 8 Experiments of Muniz et al. (2007) on static field assisted *Saccharomyces cerevisiae* biomass growth. (A) Experimental system – schematically. (B) Biomass and glucose evolution in time.

glucose level in the magnetized culture attained a level of 32.2% greater than that in the non-magnetized culture.

As a segment of these experiments, Motta et al. (2001) performed a test at 110 mT, 220 mT and 350 mT field exposures under the same experimental conditions (vessel volume and speed of stirrers). The results can be summarized as follows:

- The non-exposed cell cultures exhibit a growth greater than those of the exposed yeast.
- The exposed cell at 110 mT culture resulted in a lower biomass development than the non-exposed cell for the first 12 h.
- The second 12 h of the inoculations revealed a magnetically induced growth. The cultures exposed to 220 mT showed three peaks of the absorbance (spectrophotometric measurements at 610 nm wavelength light), whereas at 110 mT only two peaks appeared.
- The produced CO₂ volume showed higher pressure values in the exposed samples than the control samples. After 8 h inoculation, at 110 mT, the released gas was only approximately 7%, whereas at 220 mT the gas was 21.3%, more than the control volumes. These results are shown in Figure 9.

In general, Motta et al. (2001) and Muniz et al. (2007) explain the observed magnetic stimulation of the process on the basis of interpretations of Blanchard and Blackman (1994) attributing the holistic effect in the yeast performance to magnetic effects on certain proteins, more specifically to enzymes.

Anton-Leberre et al. (2010) clearly claim and confirm by results that high static and pulsed magnetic fields do not affect the cellular processes in the *S. cerevisiae* yeast. Some principal results of this study will be discussed next. These authors provide a comprehensive overview of the existing results about high-intensity magnetic field exposures of living organisms and particularly focus on the contradictory results. To test the main concept about the high-field intensity effects on *S. cerevisiae* yeast experiments were performed in two magnetization modes: a short-time exposure (30 min) to a static field of 14 T and a long-time exposure (8 h) to 16 T.

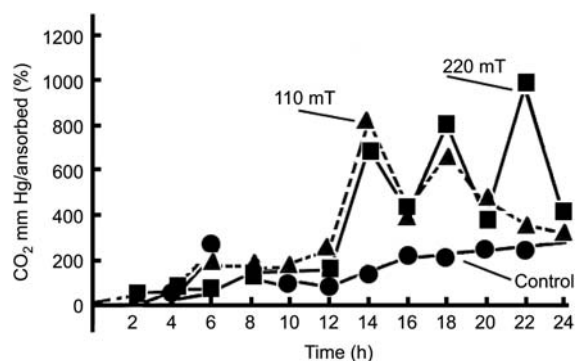


Figure 9 Ratio of the average of the produced CO₂ pressure (mmHg) and *Saccharomyces cerevisiae* culture biomass (RCA) for two exposures and 24 h fermentations. Adapted from Motta et al. (2001).

Additionally, yeast cultures were exposed to a pulsed (sub-seconds) high-intensity magnetic field (37 T and 55 T) as well as to 20 T field pulses repeated one to four times.

Upon such conditions, the investigations referred to some specific problems, including:

- Genome-scale protein expression experiments performed with long-term exposure (14 T) and pulsed magnetization (37 T) did not reveal any effects on the transcriptional machinery in yeast. The experiments were repeated with stronger fields: 16 T for 8 h and a single magnetic pulse of 55 T. The authors suggested that the absence of gene expressions in yeast exposed to high magnetic fields does not exclude effects on the cellular function at the proteomic level. The test was performed by 2-Delectrophoresis (Boucherie et al. 1995, Perrot et al. 2007). The results for all field exposures were almost similar, thus it was decided that protein expressions were not modified by magnetizations.
- Viability and cell morphology were tested through coloring cells by methylene blue because in viable yeast cells the enzymatic reactions oxidize the blue indicator into a colorless compound. The indicator allows establishing that the cells are metabolically active but not whether they are still able to divide. The test clearly indicated that the stained cultures with methylene blue and magnetized after that were practically the same as those of the control samples (non-magnetized): the greater part of exposed cells turned white. Because the cell content is diamagnetic, the high magnetic fields can create torques leading to endogenous migration of cellular material as a result of differences in the diamagnetic characteristics. The image processing technique CalMorph (Ohtani et al. 2004) was used to obtain changes in cell shape, bud distribution, wall thickness, position and shape of nucleus, etc. Statistically significant results from 200 individual cells exposed to four pulses of 20 T definitively revealed that the cell morphological parameters of the exposed culture and the control samples are identical.
- Growth and fermentation activity in exposed cultures under 4×20 T pulses in rapid sequence were monitored up to 6 h (samples at every 30 min) in a rotary shaker. No significant effects on both the growth rate and the rates of ethanol and glycerol production were detected (Figure 10).

In the context of high static magnetic field effects on the proliferation of *S. cerevisiae*, we have to mention the detailed studies of Ikehata et al. (2003) and Iwasaka et al. (2004), both of them performed under high fields up to 14 T. These authors observed deceleration in the cell growth and this effect was reproducible in all runs of proliferation. Furthermore, the gas (CO₂) produced by the cell decreased at high magnetic fields of the order of 10 T. Furthermore, in a gradient magnetic field with a vertical orientation (parallel to the gravity), the field effect on the cell proliferation was also towards deceleration. Moreover, they suggested some mechanisms explaining the inhibitory effect of the field, namely Iwasaka et al. (2004), briefly outlined next:

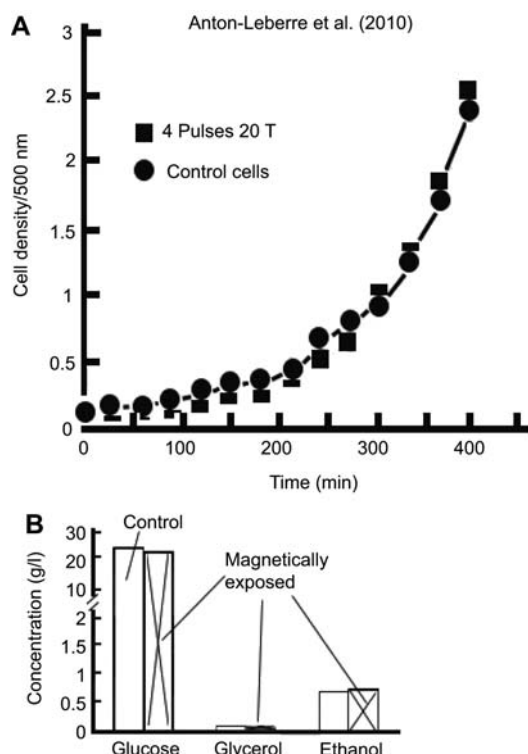


Figure 10 Experiments of Anton-Leberre et al. (2010). (A) Growth curve. Reinoculated after the exposure in YEPD (yeast extract peptone dextrose) medium and cultured at 27°C. (B) Concentration of residual glucose, glycerol and ethanol produced in yeast exposed to four pulses of 20 T and control cells.

- The oxygen is consumed by the cells and they produce CO_2 . The concentrations of paramagnetic oxygen molecules and diamagnetic carbon dioxide molecules change during the yeast proliferation. The total magnetism of the yeast culture system changes towards diamagnetism.
- It is considered that a concentration gradient of oxygen molecules occurs in the solution because the oxygen concentration beneath the air-solution boundary is higher than in the bottom part of the vessel. When the gradient of magnetic susceptibility is in a vertical line, the magnetic field gradient in the horizontal direction causes a convectional flow which increases the oxygen concentration around the yeast, thereby enhancing the proliferation of the yeast. This the so-called Mechanism-1 suggested by Iwasaka et al. (2004) is illustrated in Figure 11B. However, the results indicated that the gradient magnetic fields decelerated the proliferation of the yeast.
- Another speculation is that a multiplication of hydrostatic forces and diamagnetic forces affected the proliferation of the yeast. Magnetic forces acting on diamagnetic water molecules are converted into hydrostatic forces. The parting of water by magnetic fields changed the altitude of the water by 23 mm at 8 T (Ueno and Iwasaka 1994) and 60 mm at 14 T, increasing the hydrostatic forces in the water. The yeast conversion of paramagnetic oxygen to diamagnetic carbon dioxide gases decreases the paramagnetic forces of the oxygen. The exponentially proliferating

yeasts generate high pressure of the CO_2 gas. The diamagnetic force acting on the condensed CO_2 gas adds to the existing one and acts on the yeast sedimentation, as illustrated in Figure 11C, explaining Mechanism-2 of Iwasaka et al. (2004).

- Even though the two mechanisms of Iwasaka et al. (2004) are reasonable, they do not support the observed effects of the magnetic field on the cell proliferation.
- Mechanism-3 suggests the behavior of paramagnetic oxygen molecules under a spatial gradient magnetic field (Figure 11D) and also refers to induced convection by paramagnetic oxygen molecules and gradient magnetic field.

In conclusion, yeasts in a liquid-gas mixture system under gradient magnetic fields (14 T) exhibited decelerated growths. Iwasaka et al. (2004) attributed those negative effects to the field disturbed mass transfer process, especially to the transport of the paramagnetic oxygen gas and the diamagnetic materials (carbon dioxide, water, etc.). According to Iwasaka et al. (2004), the main effect comes from the decreased oxygen supply.

We especially referred to the mechanisms of Iwasaka et al. (2004) explaining the field effect on the cell proliferation because they will never work if a real fermentation with a mechanical mixing is carried out. In addition, these mechanisms can work in strong fields capable of controlling the motion of diamagnetic and paramagnetic elements in the liquid phase, but such mechanisms can never occur in low fields and liquid media with increased viscosity such as those in the real fermentations.

3.2.2.2. Comments on the proliferation studies: micro-level dynamo concept The results presented on *S. cerevisiae* proliferation in magnetic fields reversal claim that either the exposures inhibit the development (Mehedintu and Berg 1997) or there is no effect on the cells (Anton-Leberre et al. 2010). The results of the latter studies confirm those of:

- Ruiz-Gomez et al. (2004), with field of 0.35–2.5 mT and 50 Hz.
- Nakasono et al. (2003), with field of <300 mT and 50 Hz for 24 h.
- Ikehata et al. (2003), with a static field up to 14 T, where 5900 genes were examined.
- Iwasaka et al. (2004), with a static field up to 14 T and observations on cell proliferation.
- Binniger and Ungvichian (1997), with 60 Hz, 20 μT with no effect either on the genes expression or cell division.

The missing magnetic field effect was attributed to the fact that in the dominating cases it was studied in the stationary phase of growth where the cells have already attained resistance mechanisms to stress (Anton-Leberre et al. 2010). The failure to find magnetic field effects in most of the experiments is mainly due to this preliminary defined condition. The experiments of Anton-Leberre et al. (2010) were conducted in the exponential phase with not yet developed resis-

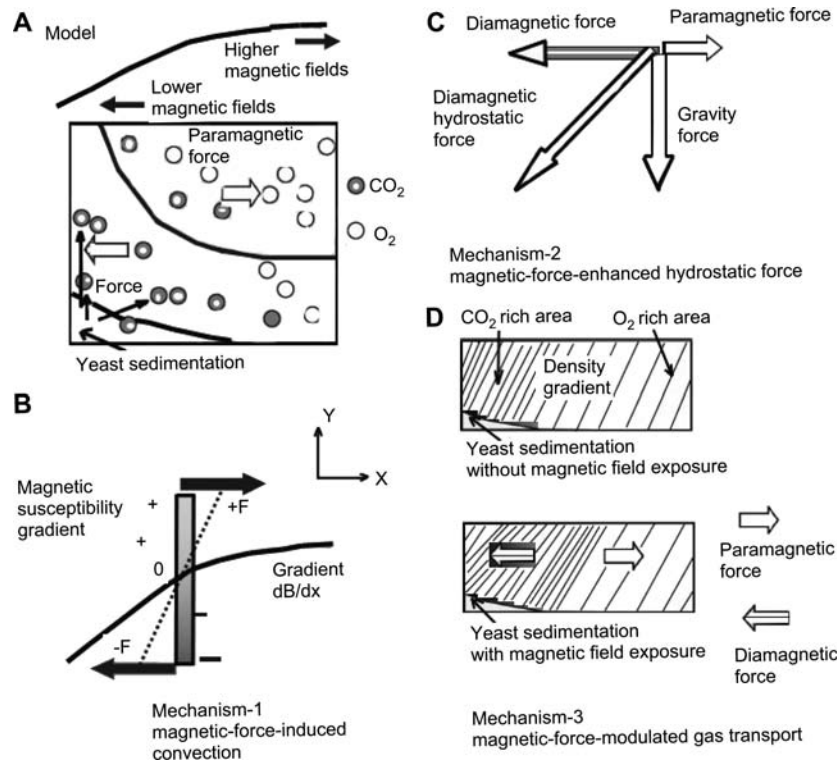


Figure 11 Mass transfer models of Iwasaka et al. (2004): see the text for explanations. Adapted from Iwasaka et al. (2004).

tance mechanism of the cells, no limitation to the nutrient supply and an active phase of growth.

An important parameter is the energy absorbed during the magnetic field exposure. In static fields, in general, there is no field energy transmission to the living cells, but the field can alter the energy levels of certain molecules (Rogers and Hore 2009, Anton-Leberre et al. 2010). However, pulsed and time-varying fields (mainly 50 Hz in this analysis) induce eddy currents in the aqueous culture media, which are highly conductive due to additional ions added. These eddy currents generally produce heating in the culture medium and the cell cytoplasm. However, we have to recall that the penetration of the field-induced eddy currents is limited to the surface of the liquid only, the so-called “skin effect”, which means that only those cells which are close to air-culture interface are affected. The eddy currents affected zone decays rapidly in depth with increase in both the field magnitude and the frequency. With high-intensity magnetic pulses (Anton-Leberre et al. 2010), the field cannot penetrate the sample in depth in contrast to the case where only static field is applied.

In addition, the eddy currents create their own magnetic field that in a co-action with the principle one (that used for the exposure) creates small dynamos mixing the liquid at micro-level. Such microscopic dynamos can enhance the mass transfer at the vicinity of the cell surfaces where stagnant liquid layers exist and the mass transfer is totally controlled by diffusion, irrespective of the intensity of mechanical mixing. Because, in most of the proliferation experiments, the mixing was not intensive and the cell

growth was already accomplished, it might be expected that no field effects boosting the cell living activity were found. Any suppression effects (Medhindu and Berg 1999) might be attributed to the local heating that alters the conditions of normal cell growth, but in general, it cannot stop the process due to rapid heat dissipation in the medium and in the vessel.

In contrast to the above mentioned results (Mehedintu and Berg 1997, Anton-Leberre et al. 2010), the experiments of Motta et al. (2001) and Muniz et al. (2007) show just the opposite tendencies, i.e., stimulated biomass growth in exposed cultures. Hence, the principle questions is: Are the experiments of Motta et al. (2001) and Muniz et al. (2007) correct? What is the reason for such different results? If we refer to the above mentioned hypothesis of the micro-level dynamos (MLDs) created by induced eddy currents, we can comment on the contradictory results from a common point of view, namely:

- The exposure of a static conduction medium to time-varying magnetic fields (pulsed or AC) or agitation of the same suspension in a static magnetic field, from the point of view of eddy current generation, is one and the same, because there is a relative motion and variation in time of the medium and the magnetic flux lines: this is the basic Faraday law of generation of electricity.
- Hence, we can suggest that the MLDs working in the vicinity of the cell-liquid interface enhance the mass transfer by reducing the diffusion mass transfer resistance, i.e., there is an increase of the nutrient flux to the cells, which without any changes in the morphology and the

metabolism augments their growth rate. From this point of view, the experiments of Medhundu and Berg (1999) in the “positive windows” (with positive field effects) in accordance with their terminology are close to those of Motta et al. (2001) and Muniz et al. (2007). One can recall in both cases the field intensity is low and the frequency, too, thus the eddy currents can penetrate the liquid in depth and the MLDs will be at work.

- When strong static fields are applied (Anton-Leberre et al. 2010) no eddy currents emerge, except at the very beginning when the field is turned on. Then, the field has no effect on the transport processes in the stagnant liquid. If the field is strong and pulsed or static, and the liquid is agitated, the eddy currents are induced in a very thin layer at the surface of the medium and do not penetrate into the bulk, this is the so-called “skin effect”. Hence, the MLDs cannot affect the transport process in bulk of the cell suspension. Therefore, without any effects on the morphology and the cell metabolism, and the mass transfer in the culture medium, as well, the exposed cell growth will be, in fact, as that exhibited by the non-magnetized suspension. In fact, this confirms all the results of Kimball (1937) where 11,000 Gauss in solid medium (hence there are motion and eddy currents) impeded the yeast growth by approximately 20–30%. Similar results were reported by Jennison (1937) with bacteria and yeast less than 3000 Gauss static magnetic field in a Petri dish.

The hypothesis of MLDs conceived here refers to the liquid-side mass transfer in the cell growth process and to only mechanical effects in the liquid phase. The MLD concept does not touch the intracellular process and tries to arrange the existing experimental facts into a common structure. It provides explanations of the magnetic field effects within the framework of the macroscopic (extracellular) mass transfer processes, without any associations with “magic magnetic effects”. The effects on the cell membranes are also encompassed by the MLD concept, but the existence of electric currents near the cell (eddy currents) with their own magnetic fields and potentials suggests effects on all phenomena mentioned above, as C^{2+} influx, ion activity, enzyme turnover, etc. It is important to note that all these effects take place at the near-field range, at the vicinity of the cell-membrane interface. Hence, the magnetic field effects have to be looked for at this location irrespective of the scale of the magnetic field application: over Petri dishes or in large magnetically assisted bioreactors. In this context, recall the models of Iwasaka et al. (2004) also concerning magnetically controlled transport processes outside the cell. However, the proposed physical mechanism of the MLD is somewhat different and works at a micro-level near the cells in contrast to the models of Iwasaka et al. (2004) explaining the macro-mixing in the system.

In the context of some biological effects as a result of the exposure to magnetic fields, Liboff (1969) is referred to who discusses the Larmor precession relevant to the intracellular diffusion processes. Liboff (1969) calculated that the minimum field intensity required to provoke some biological effects is approximately 10^{13} Gauss; for example, $1\text{ T} = 10^4$

Gauss, which is impossible to be created in a conventional biotechnological laboratory. Hence, other effects have to be looked for that could control the cell growth, rather than mythic “magnetic intracellular effect” and explain the observed results.

The MLD concept will be further mentioned in this review for explanation of the magnetic field effect on living cultures together with other boosting or inhibitory mechanisms suggested in the literature.

3.2.2.3. Fermentation processes In contrast to the proliferation studies searching magnetic field effects at micro-levels, the performed fermentation studies directly copied the traditional fermentation process of ethanol fermentation (Cardona et al. 2009) in vessels entirely exposed to external magnetic fields (Motta et al. 2001, Muniz 2002, Muniz et al. 2007) (Figure 8A). Two 200 ml reactors (control vessel and magnetically exposed) simultaneously performed ethanolic fermentation. The magnetic field exposure for the stimulated fermentation was established at 550 mT. The results were taken from eight identical runs by continuous monitoring of ethanol and glucose concentration, and of the yeast growth. The time evolution of average values based on all runs are shown in Figure 12A,B. In general, the ethanol production and the glucose uptake are augmented in the magnetized culture by 30% and 20%, respectively. The glucose consumption was almost complete at the end of the magnetically assisted fermentation run in contrast to the control (non-magnetic) counterpart. Furthermore, the rates in the ethanol production and the yeast growth attained values of 3.4 and 3.0 times greater than those in the control fermentation runs. In this context, the ethanol yield per gram of glucose, per gram of biomass reached values of 4.2 times greater than those in the unexposed runs. These results are supported by the glucose/biomass ratio which was only 1.17 times higher than in the control fermentation. The differences in the parameters and values representing both the exposed and the control runs are shown in Figure 12C.

Motta et al. (2004) clearly indicated that the significant changes in the performance of the exposed fermentation were observed after an initial lag phase of 4 h up to the end (24 h) of the process. This supports the hypothesis of efficient field effect on the yeast growth and ethanol production in the exponential phase. The final ethanol average content was approximately 2.5 times higher than in the control runs; the biomass content was approximately 2.4 times higher.

Motta et al. (2001, 2004) as well Muniz (2002) and Muniz et al. (2007) are, in fact, different reports with data taken from one series of experiments. These authors clearly denote that while in the unexposed fermentation the ethanol can alter the transport in the cell membranes and unfold the cytosolic globular enzymes (Lopes-Dahabada and Sola-Penna 2001) hindering the cell growth and proliferation, this was not observed in the magnetized yeasts. The boosting effect of the magnetic field exposure was not explained with another hypothesis of potential mechanism at the micro- or macro-level. However, if the results are looked at from the position

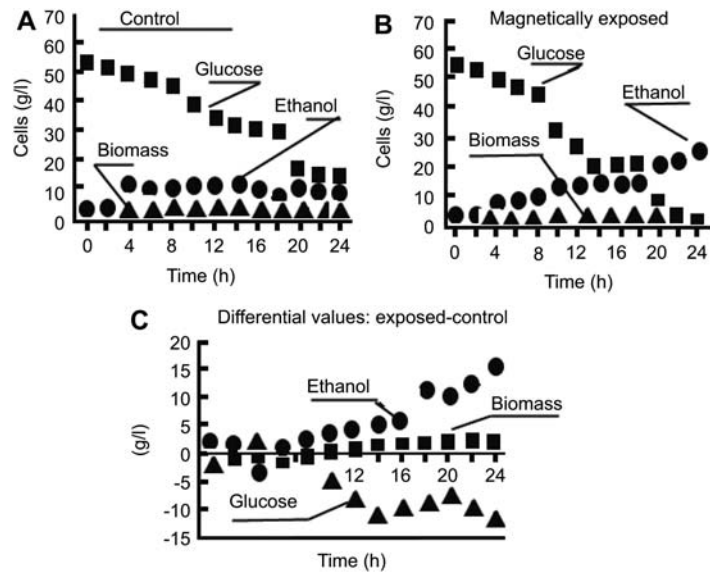


Figure 12 Fermentation experiments of Motta et al. (2004). Adapted from Motta et al. (2004).

of the MLD concept the positive outcomes of the magnetically exposed fermentation can be explained, namely:

- The inhibitory effect of the ethanol on the *S. cerevisiae* reproduction is the same in both the exposed and the unexposed processes because the macroscopic mixings created by the agitators lead to almost equal conditions allowing the ethanol to be removed from the vicinity of the cell surfaces.
- With macro mixing, this can be attained by increasing the rotation of the stirrer to some extent, but at the vicinity of the cell surface there is a thin liquid layer which is almost stagnant and close to saturation with regard to the ethanol released by the cell. Hence, this layer becomes the main mass transfer resistance, namely, with regard to the nutrients from the liquid to the cells and from the cells to the liquid with regard to the metabolic products as well.
- Hence, the increased nutrient flux towards the cells is augmented by the micro dynamos that with unchanged cell metabolisms enhance their growth and production.
- Moreover, the micro mixing due to micro dynamos at the vicinity of the cell-liquid interface augments the removal of the ethanol, unlike the case of the unexposed process where this removal is controlled by pure molecular diffusion only. Hence, the ethanol inhibitory effect at the vicinity of the cell-liquid interface can be reduced to some extent.
- In addition, we can only suggest, without any data at the moment, that eddy currents at the vicinity of the cell walls can stimulate the transport across the membranes as mentioned above in accordance with dozens of hypotheses existing in the literature. However, this suggestion needs further experimental verifications.

The recent study of Santos et al. (2010) considered static MFA biomass and glutathione (GSH) production (Figure 13). The field was induced by a Helmholtz pair (10 cm ID and

18 cm external diameter) surrounding the entire fermentation vessel (250 ml). The field intensity for exposure was created with the range 25.0–34.3 mT; no explanation about the choice of the field intensity was published. In general, there is no originality in this study except the product (GSH), of course. The data are difficult to analyze because no direct experimental information is provided. The main approach is to present outcomes of planned experiments treated by analysis of variance (ANOVA). This simply means a low number of experiments (only seven runs) behind the studies and problems to extract tendencies in the biological system behavior when a magnetic field is imposed. The quantitative results obtained for runs lasting 24, 48 and 72 h, and field exposures from 8 to 16 h range (of each run), reveal that:

- a. The biomass results (Figure 13A) (first point taken after 24 h of incubation) show field effects induced augmentations with regard to the control run.
- b. The higher biomass concentration (16.26 g/l) was obtained with a 72-h fermentation run and the longest field exposure time of 16 h at 25.0 mT. Similarly, in a run with exposure time of 8 h, and 25.0 mT, the biomass was almost the same, 16.11 g/l. The authors attributed the differences to the time of the incubation period (72 h and 48 h, respectively), but based on the previous comments in this section of the review it should be mentioned that the time for exposure coincides with the exponential phase. Therefore, the results are similar to those in other studies commented here, for example, those of Motta and colleagues.
- c. With regard to the GSH yield (Figure 13B), a stimulatory effect was observed in all the runs except those exposed for 8 h and 12 h in fields of 34.3 mT and 29.6 mT, respectively. The specific GSH yield varied from 15.68 to 20.91 mg GSH/g biomass at 72 h of incubation: the highest value was obtained with the longest exposure

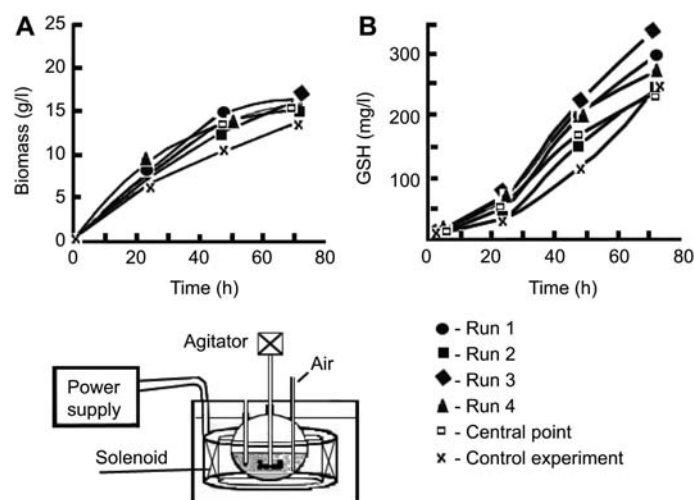


Figure 13 Experimental results of Santos et al. (2010) on glutathione (GSH) production by magnetically assisted *Saccharomyces cerevisiae* fermentation. (A) Biomass growth. (B) GSH production in time.

time (16 h) and the lowest magnetic field induction (25.0 mT). In any case, these are facts that are difficult to prove or use for a systematic analysis.

3.2.3. *Spirulina* behavior under magnetic fields The development of autoregenerative biological life support systems for men in space or under terrestrial conditions based on photosynthetic organisms, such as higher plants and algae, providing edible material (Morist et al. 2001, Colla et al. 2007, Harun et al. 2010) and fuels (Greenwell et al. 2010, Khan and Rashmi 2010, Kumar et al. 2010, Mutanda et al. 2011) are of primary importance for the future sustainable development of human life. In this context, the position of *Spirulina* is important due to its relevance to production of either foods or fuels because it can be used in continuous working photo-bioreactors (Morist et al. 2001, Greenwell et al. 2010). *S. platensis* as a representative of the *Spirulina* family and will be commented regarding the magnetic field effects on its growth.

Spirulina platensis is a somewhat quality health food with many attempts applied to optimize the cultivation process. Earlier attempts to stimulate *S. platensis* growth was performed by Hirano et al. (1998) in a static magnetic field with intensity ranging from 50 Gauss up to 700 Gauss and a terrestrial magnetic background of 0.5 Gauss. The optical density and the dry weight method were used to evaluate changes in *S. platensis* growth. In accordance with the results of Hirano et al. (1998), the field effect on the growth was positive within the range from 50 to 350 Gauss and the highest was observed at 100 Gauss (Figure 14A). At 100 Gauss the specific growth rate was 0.22 d which was 1.5 times higher than that observed under the earth magnetic background of 0.5 Gauss.

Further increase in the field intensity within the range 400–700 Gauss led to inhibitory effects and the specific growth rate decreased to 50% of that observed under the earth magnetic background. Hirano et al. (1998) found out

that the O_2 evolution rate during the photosynthesis was 100 and 240 $\mu\text{mol } O_2$ [i.e., (mg chlorophyll)/h] at 0.5 Gauss and 100 Gauss, respectively. Moreover, the sugar content in *S. platensis* was higher at 100 Gauss, attaining a level of 178 mg/(g dry cells), which is approximately 20% higher than under the earth magnetic field. Beyond the point of 100 Gauss, the sugar content decreased down to levels of 60% and 50% of those obtained at 0.5 Gauss and 100 Gauss, respectively.

The positive effect on the growth rate, sugar content and O_2 evolution rate directly indicates that in magnetically assisted cultures the light excitation of chlorophyll, production of radical pair, and the conversion of light energy to chemical energy are augmented. The inhibitory effects were attributed by Hirano et al. (1998) to magnetic field suppressive action on sugar synthesis from carbon dioxide.

To investigate whether magnetic fields affect sugar synthesis, Hirano et al. (1998) performed a heterotrophic culture (*S. platensis*) that revealed no impact of the magnetic field on *S. platensis* growth (Figure 14B). Moreover, these results directly show that the field has no effect on secondary metabolites when the intensity is lower than 700 Gauss.

The content of photosynthetic pigments in *S. platensis* cultured at 100 Gauss was 148 mg/(g cell) which was an increase of approximately 54% beyond the level observed at 0.5 Gauss. The strongest magnetic field used in these experiments (700 Gauss) inhibited the process and the amount of photosynthetic pigments dropped down to 20% of that under the earth magnetic background. These results are illustrated by Figure 14C,D. In this context, the glyceroglycolipid content [mg/(g dry weight cell)] was affected in a greater extent by the magnetic field applied: 48.4 (0.5 Gauss), 40.5 (100 Gauss) and 21.0 (700 Gauss). Based on these results, Hirano et al. (1998) suggested that glyceroglycolipids play some role in the reaction sites of phycocyanin.

Similar effects were reported by Li ZY et al. (2007) in periodically magnetized *S. platensis* cultured in an exter-

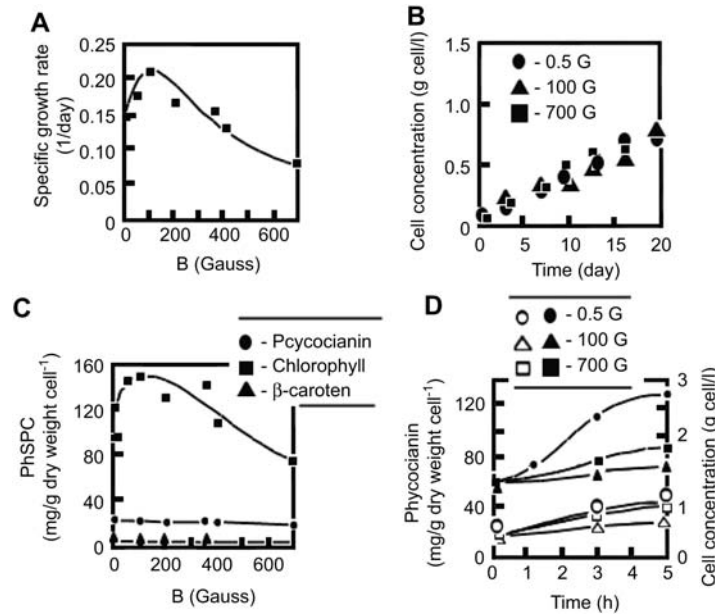


Figure 14 Magnetically exposed *Spirulina platensis* growth. Adapted from Hirano et al. (1998). (A) Field effect on the growth rate in the culture. (B) Time course on growth in heterotrophic cultures of *S. platensis*. (C) Field effect on the content of phycocyanin, chlorophyll and β-carotene in the culture. (D) Time course of the phycocyanin content in the *S. platensis* culture and the cell concentrations at different field exposures.

nal lop airlift. These results are commented in detail in section 3.3 “Reactors with recirculation and external culture magnetization”.

3.2.4. Magnetotactic bacteria (*Magnetospirillum magneticum*) Magnetotactic bacteria (MB) are Gram-negative prokaryotes that synthesize intracellular magnetic nanoparticles, termed “magnetosomes”, which are membrane-bounded crystals aligned in the chain-like structures within the cell (Blakemore 1975, Scheffel et al. 2008). Magnetosomes are of great interest as potential carriers (Schüler and Frankel 1999, Matsunaga et al. 2007). MB synthesize unique intracellular structures called magnetosomes, which consist of biogenic magnetite (Fe_3O_4) or greigite (Fe_3S_4) and allow to orient themselves in geomagnetic fields, possibly to enable a more efficient search for an optimum microaerophilic environment (Pan et al. 2009).

Magnetospirillum magneticum was investigated by Popa et al. (2009), with regard to the oxidative stresses on the growth of intracellular magnetic particles. Cell growth of MB was monitored by cell density (cell/ml) using a spectrophotometer. The first issue was the effect of O_2 on the growth rate. It was estimated that from zero up to 147 μM initial O_2 (aq) range, stirring the culture at high O_2 resulted in faster exponential growth of cells and larger cell densities in the stationary phase (up to $\sim 5 \times 10^8$ cell/ml; ~ 0.28 g dry weight/l after 72 h). The growth was inhibited above 13.2% initial O_2 (g) [i.e., $> 147 \mu\text{M}$ O_2 (aq)] in stirred cultures. The inhibition was attributed to oxidative stresses because the magnetite growth requires $\text{Fe(III)}:\text{Fe(II)} \sim 2:1$ ratio, whereas chemical ion oxidation is fast and has a high equilibrium

constant at neutral pH (Matsunaga et al. 1991, Heyen and Schüler 2003). The results are illustrated in Figure 15.

The *Magnetospirillum* sp. strain AMB-1 was subjected to magnetization experiments by Pan et al. (2009) and cultured at 28.8°C in an enriched magnetic spirillum growth medium at $\text{pH}=6.75$ (Yang C et al. 2001). The culture optical density at wavelength of 600 nm was used to estimate the cell growth rate. The cellular magnetism was measured by a spectrophotometer using the Zhao et al. (2007) method. A pulsed magnetic exposure was performed by two solenoids (experimental coil and control coil): 200 mm ID and 400 mm

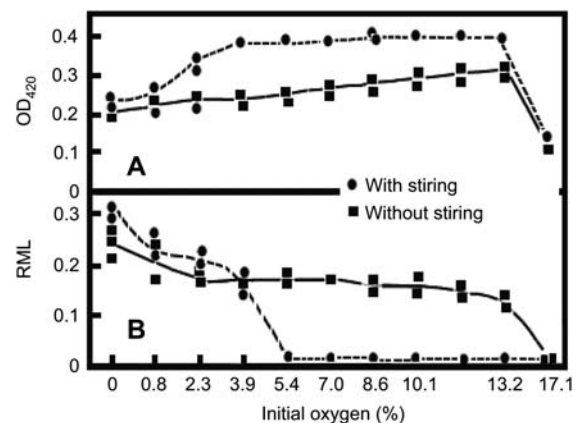


Figure 15 Effect of the oxygen on *Magnetospirillum magneticum* strain AMB-1 under two mechanical conditions. Adapted from Popa et al. (2009). The initial O_2 values are concentrations in the gas phase at 1 bar. (A) Oxygen effect on the growth. (B) Oxygen effect on the magnetite content RML.

in height (Figure 16A). Each coil was made up of two sub-coils that produced the same magnetic strength but connected in different order in series: a) when the currents in the two subcoils are in the same direction, the field is generated by the experimental solenoid (coil); b) when the currents in the two subcoils are opposite, then, the resulting field was close to zero. This was the control coil. The field was generated by 50 Hz current with 50% duty (one pulse per period of the current) at 2 mT (20 Gauss strength, Figure 16B).

Magnetic precultures were prepared by inoculating the wild-type *Magnetospirillum* sp. strain AMB-1 microaerobically at 28.8°C and used at the exponential growth phase. The magnetic bacteria AMB-1 was exposed to a pulsed magnetic field at 28.8°C for 24 h. The growth is rapid into exponential phase after inoculation, reached a peak value oxygen demand at 8–12 h and remains steady during the following 12 h (Figure 16C). No difference in the growth behavior of both the magnetized and the control samples were detected.

The remanent magnetizations (R_{mag}) of both samples decreased rapidly to a level of 0.65 after 2 h of inoculation (Figure 16D). The effect was attributed to a delay in the synthesis of magnetosomes as a result of intensive cellular growth and division: a temporary effect induced by the increase in the amount of dissolved oxygen. The remanent magnetizations of both samples increased slowly beyond a 4-h inoculation and attained almost steady level of 0.9 16-h of the run; it remained until the last measured time point at 24 h of the process. Hence, the field induced growth was significant, because in the control volume the remanent magnetization varied slowly in time with a rate slower than that of the magnetized culture and reached a level of approximately 0.75.

The experiments of Pan et al. (2009) reveal that the pulsed field magnetization augments the cellular magnetism in *Magnetospirillum* AMB-1 with slightly increased ratios of superparamagnetic particles (<20 nm) and mature magnetosomes (>50 nm). Moreover, the results indicate that lower strength of pulsed magnetization practically does not affect the bacterial cell morphologies but can boost both the biomineralization of magnetosomes and the magnetosome production. These latest results confirm the earlier experiments of Ricci et al. (1991) that were performed under similar conditions but at stronger fields (45 and 65 mT) where the wild-type morphology was saved, but higher fields (80 mT) led to larger amounts of (coccoid) cyst-like cells and abnormal arrays of magnetosome chains.

In the context of the magnetic field effect on the growth of cellular magnetism, it is noteworthy to comment on the experiments of Sun et al. (2008) with *Magnetospirillum gryphiswaldense* MSR-1 used as a model organism to establish optimal methods for supplying substrates, and for increasing magnetosome yield when the cultivated cells are supplied solely with air. These experiments were performed without assistance of a magnetic field, but could serve as a good basis for future experiments in view of the fact that they show the optimal non-magnetic conditions for magnetosome growth.

3.2.5. *Escherichia coli* behavior under magnetic field

Escherichia coli is a bacterium widely investigated for magnetic field effect during its growth (Tsuchiya et al. 1996, Fojt et al. 2004, Gaafar et al. 2008). Two main approaches exist in the literature: a) static, high-intensity field exposures and b) low-intensity, time-varying fields.

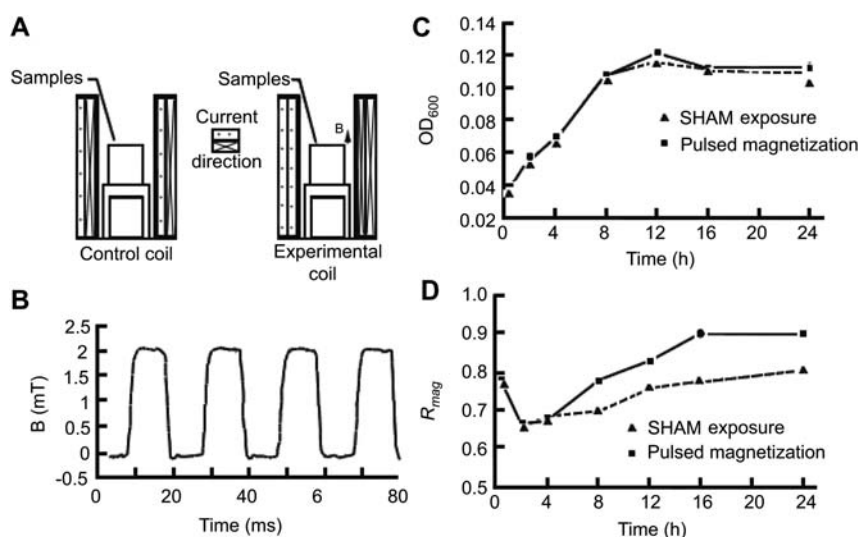


Figure 16 Experiments of Pan et al. (2009) on pulsed magnetization exposure of *Magnetospirillum* sp. strain AMB-1. (A) Experimental system – schematically. (B) Magnetic flux density (induction) at the solenoid center. (C) Growth curves of *Magnetospirillum* sp. strain AMB-1. (D) R_{mag} as a function of the time; $R_{mag} = \left(\frac{OD_{\uparrow} - OD_{\Rightarrow}}{OD} \right)$; $OD_{\uparrow}$ is the value for parallel magnetic field; $OD_{\Rightarrow}$ values for transverse magnetic field. OD – optical density defined under normal (without magnetic field) conditions. Note: SHAM exposure = a control group used to simulate the environmental conditions of exposed samples, but in the absence of exposure.

The results from these studies are somewhat different and opposite in viewpoints. They will be discussed separately which will allow us to draw the main tendencies and to find probable explanations of the results.

3.2.5.1. Static and high-intensity field exposures Tsuchiya et al. (1996) cultivated *E. coli* in both homogeneous and inhomogeneous magnetic fields with inductions from 0.5 T up to 7 T employing a superconducting magnet of 16 cm poles (in a diameter) and a shaking flask (50 ml). The experiments performed reveal that in the stationary phase the viable cell count decreased but the ratio was larger than 1.0. Hence, under high magnetic fields the cells are less likely to die. Survival of the cells was more pronounced under inhomogeneous magnetic field (Figure 17B–D,F) compared with homogeneous magnetic field (Figure 17A,B). Moreover, the growth patterns observed in the initial 3–6 h incubation time (Figure 17B,D) in 7 T homogeneous and 5.2–6.1 T inhomogeneous fields revealed that the cell number was less than 1.0 indicating field effects on the initial growth of the bacterium.

Tsuchiya et al. (1996) suggested that a super high magnetic field causes stress to the cell taking into account that the extracellular nutrients and chemical conditions in batch culture are constantly changing. The latter suggestion gives rise to metabolic differences between exponential and stationary phase cells and consequently the growth phase of the

bacterium is magnetically affected. The reduction in the mixing culture (Figure 17E,F) with regard to the experiments presented in Figure 17A,B led to a lower number of cells, mainly due to a lower amount of dissolved oxygen in the culture medium. Furthermore, the analysis of the cell morphology (by electronic microscope) in geomagnetic and high laboratory fields indicated no differences in the stationary phase. No explanations of the results obtained were provided in this article. Further work by Tsuchiya et al. (1999) is almost the same with repetitive results and conclusions.

The experiments of Horiuchi et al. (2001) show drastic suppression of *E. coli* death when high magnetic fields are exposed. Based on these results it follows that under inhomogeneous high-intensity magnetic field of 5.2–6.1 T, the number of cells in the stationary phase under the high magnetic field was 100,000 times higher than that under a geomagnetic field (Figure 18). The strain was cultivated in Luria-Bertani (LB) medium and three-times diluted LB/4 medium. It was mentioned that the addition of glutamic acid to the medium significantly accelerated cell death, glutamic acid of metabolizing enzyme could have a crucial effect on cell viability. According to Horiuchi et al. (2001), as the *rpoS* gene that encodes the RNA polymerase sigma factor plays an important role in regulating the expression of many genes in cells in the stationary phase, the expression of this protein was compared. The amount of protein expressed under the inhomogeneous magnetic field was approximately 20% high-

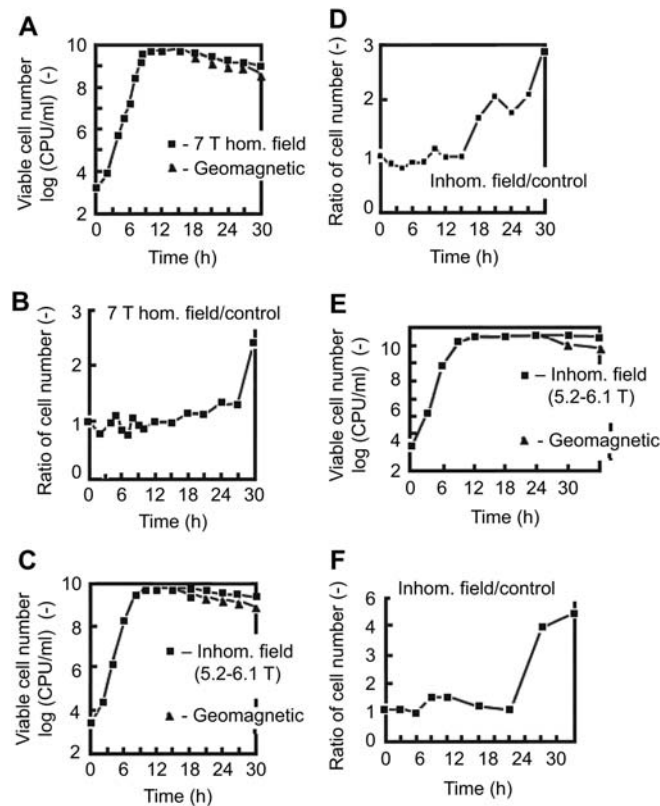


Figure 17 Experimental results of Tsuchiya et al. (1996) with *E. coli* in strong homogeneous and inhomogeneous fields. Adapted from Tsuchiya et al. (1996).

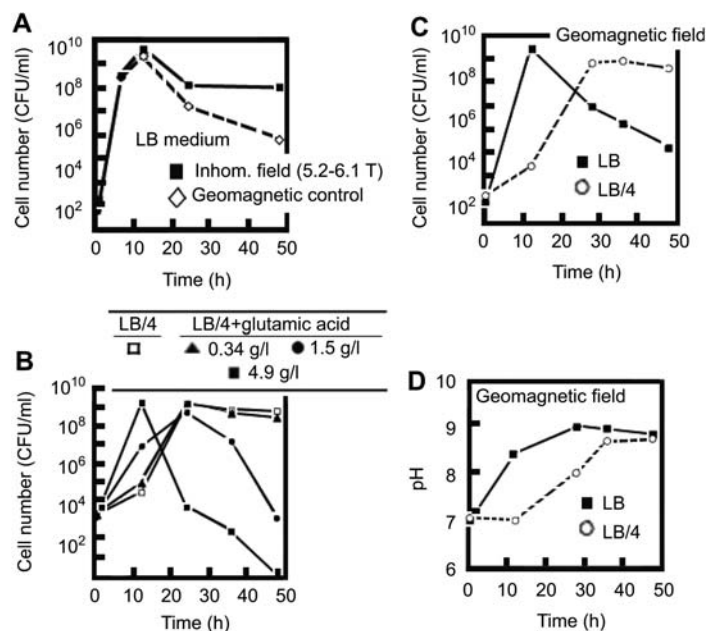


Figure 18 Drastic magnetic field suppression of *Escherichia coli* by high magnetic fields. Adapted from Horiuchi et al. (2001). (A) Effect of high gradient magnetic field of the *E. coli* cell viability in LB medium. (B) Effect of the medium on the cell viability at 43°C. (C) Effect of the medium on the cell viability at 43°C. (D) pH variations in LB medium at 43°C.

er than that under the geomagnetic field. Another study of the same group (Ishizaki et al. 2001) reported that 12-h exposure to high static magnetic field is enough to drastically suppress *E. coli* death if the field is applied after the logarithmic phase. The results are almost the same as that reported by Horiuchi et al. (2001).

The recent work of Ji W et al. (2009) addressed viability experiments with bacterial strain *E. coli* exposed to static magnetic fields. The number of colony-forming units (CFUs) was the quantitative measure of the bacterial growth. Static fields of 45 mT and 450 mT were created by magnetic disks, for fields within the range from 450 mT to 3500 mT permanent magnet (based on an yttrium alloy) was used (Figure 19A). Initially, the strain was inoculated in 50 ml LB medium for 18 h at 37°C and then 5 ml was used to inoculate new (fresh) LB medium at the same temperature for 6 h. Two identical Petri plates, control and magnetically exposed, were inoculated and monitored during the logarithmic phase of the bacterium growth after 4-h initial lag phase. The monitoring time was from 4 h until 18 h since the inoculation.

The viability test with cultures exposed to 450 mT for 60 min during the logarithmic phase (Figure 19B) led to decreased number of CFUs during the exposure period. Ji W et al. (2009) suggested that most of the bacteria were killed by the field because after the exposure, the survived cells adapted to the magnetic field (i.e., the relative number of CFUs) stopped decreasing.

The field intensity effect was the other issue in the study of Ji W et al. (2009). Exposures to all fields in the range used for 30 min at 35.8°C resulted in the data shown in Figure 19C. The authors did not explain the results but only referred to a possible effect of the ion interference mecha-

nism and the studies of Binhi (1997, 1998, 2000) and Binhi et al. (2001) and the theory of the dissociation probability of ion-protein complexes.

The next step of Ji W et al. (2009) was to see if there were any effects on the bacteria morphology due to the magnetic exposure. The analyses performed on the basis of scanning electron microscopy and transmission electron microscopy revealed that the surface of the untreated cells was smooth and complete, whereas the surface of the exposed cells was broken especially at the two ends of the cells. The authors suggested that the probable cause for the cell damage is the strong oxidation effect of oxygen free radicals produced during the magnetic exposure and referred to the studies of Brocklehurst and Mclauchlan (1996) and Kohno et al. (2000). Note that only the second reference addresses static magnetic field effects, whereas the former refers to electromagnetic fields. This indicates that the authors are not clearly aware what really happens and what is the potential mechanism of the field effect on the bacteria. The short explanations provided in this study refer to magnetic moment of rotations acting on diamagnetic anisotropic molecules of the plasma cell membranes, which as a consequence, could affect the ion permeability of the membrane channels. Hence, again the membrane channels and the field affected ion mobility are explanations because the binding state of the ion-protein complexes is changed: i.e., the equilibrium $protein(\dots) + Ca^{2+} \leftrightarrow protein(Ca^{2+})$ is shifted. Because the static field does not provoke any quantum transitions, the effects were explained by the long-life interference effect on the quantum states of the ions in the protein capsule. Furthermore, oxygen free radicals are produced during the exposure to magnetic fields and their high reactivity

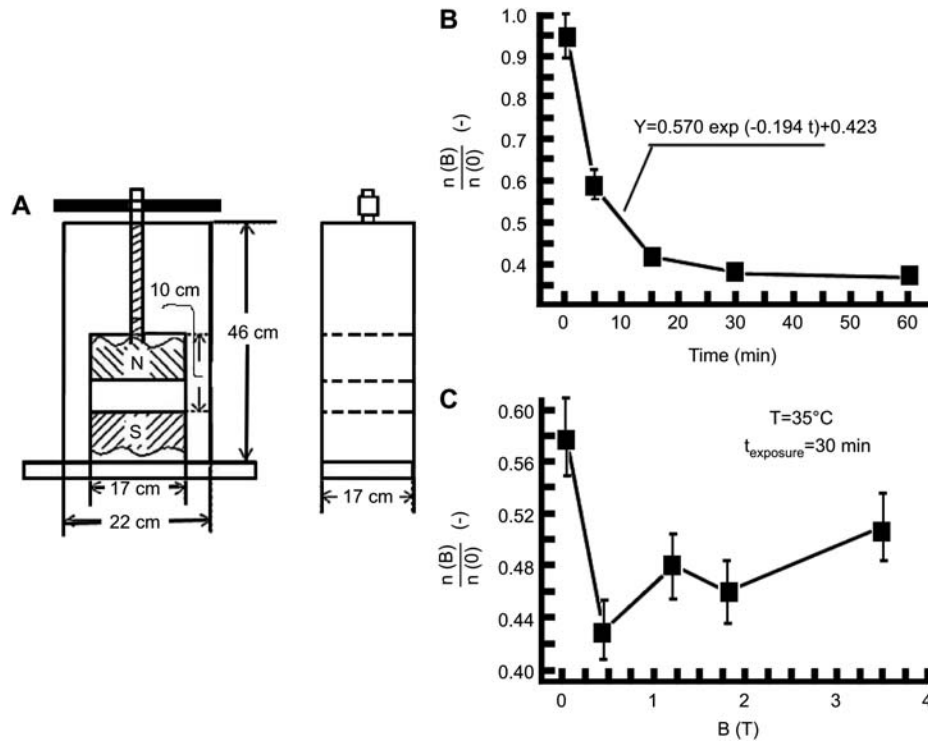


Figure 19 Experiments of Ji W et al. (2009) on static magnetic fields on *Escherichia coli* viability. (A) Experimental set-up. (B) Relative number of cells as function of time of exposure. (C) Relative number of cells as function of the magnetic field induction under limited exposure time conditions.

cause damages to the cells. These effects can take place simultaneously in the exposed bacteria and explain the results of Ji W et al. (2009).

3.2.5.2. Low-intensity, time-varying field exposures

Low-intensity and low-frequency magnetic fields are frequently used for exposure studies on *E. coli* strains with a hypothesis to find positive windows corresponding to resonant phenomena in proteins. Several representative studies and the interpretations therein explaining the macroscopic results of the field exposures will be analyzed.

Fojt et al. (2004) performed experiments with low-intensity, time-varying magnetic field (50 Hz, 10 mT) to study effects on *E. coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*. A solenoid of 205 mm ID and 210 mm height was the tool used. It was enough to create a magnetic field around Petri dishes of 80 mm in diameter. The main efforts were on the number of CFUs which were representative for the bacterial culture growth. The field was applied at the beginning of the exponential phase, approximately 4.5 h after the inoculation onset.

The exposures were only during 12 min and the amplitude of the field varied from 2.7 mT to 10 mT. The results with the magnetic bacteria are shown in Figure 20A,B. In general, there is an exponential decay in the CFUs. The strongest decrease in CFUs was conserved with *E. coli* (Figure 20A,B). The growth dynamics was investigated with exposures to a field of 60 min. The results shown in Figure 21A,B reveal a decrease in all samples, irrespective of the bacterium

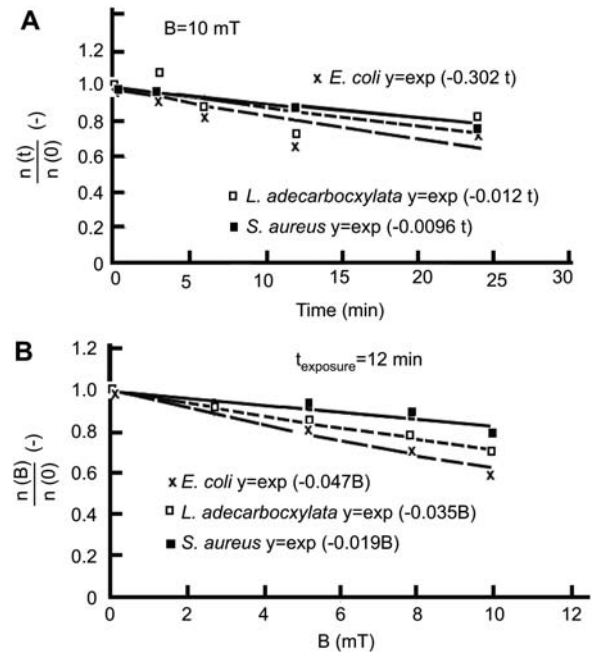


Figure 20 Low-frequency experiments of Fojt et al. (2004) on cell viability. (A) Cell viability (relative number of CFUs) as a function of the exposure time at affixed field induction. (B) Cell viability (relative number of CFUs) as a function of the field induction at a fixed exposure time.

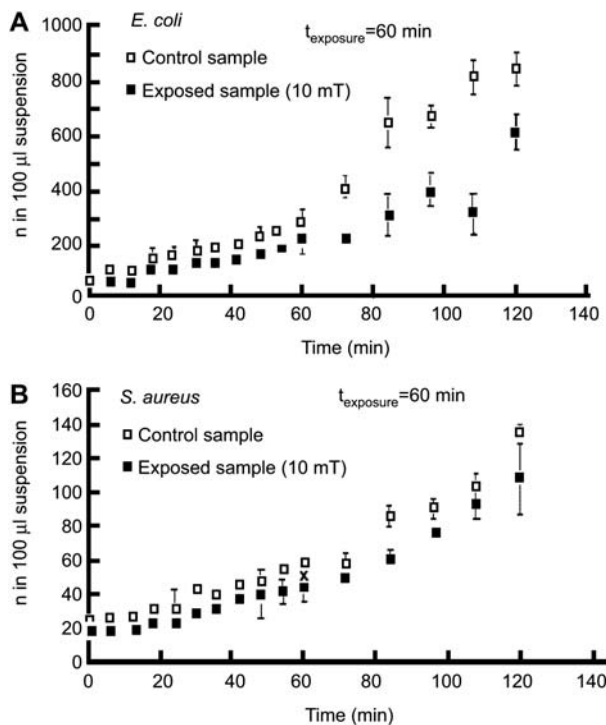


Figure 21 Low-frequency experiments of Fojt et al. (2004) on cell viability of *Escherichia coli* and *Staphylococcus aureus*.

strain. This strongly relates to the fact that a magnetic field kills bacteria (Strasak et al. 2002). It follows from these experiments that most sensitive effects were observed with *E. coli*, compared with *S. aureus*. The authors do not provide any explanation about the field induced killing effect, but on the basis of our preceding discussion we suggest that the induced eddy currents and the consequent heating kill the bacteria. This fact differs from the experiments of Tsuchiya et al. (1996), because $<50 \text{ Hz}$ AC current supply the eddy current intensity is stronger than that occurring in the shaking vessel of 50 ml exposed to strong static magnetic fields. Moreover, the penetration depth of eddy currents under 50 Hz and amplitude of 10 mT is larger than that when impulses of strong fields are applied. Hence, high-frequency and low-intensity magnetic fields kill more bacteria than high-intensity static fields. Fojt et al. (2004) tried to relate the observed effects to biological effects of electromagnetic fields, based on the possible effects on the permeability of the ionic channels in the membrane (Galvanoskis and Sandblom 1998), that is, once again magnetic field effects on intracellular processes are suggested.

In the context of the research by Fojt et al. (2004), the study of Belyaev and Alipov (2001) is referred to where low-frequency field of $21 \mu\text{T}$ (6–24 Hz) on cells of different *E. coli* K12 strain (GE499 and GE500 cells) was exposed. It was found that two resonant windows appeared with maximal effects at 9 Hz and 16 Hz. Initially, the cells were pre-incubated for 30 min at room temperature and exposed to a preset frequency for 15–20 min. A sinusoidal magnetic field

of $21 \mu\text{T}$ (30 μT amplitude) was applied by Helmholtz coils (Alipov and Belyaev 1996). The axial and transversal components of the static magnetic field were equal to $43 \pm 1 \mu\text{T}$ and $19 \pm 1 \mu\text{T}$, respectively. The method of anomalous viscosity time dependence (AVTD) (Belyaev et al. 1994, Alipov and Belyaev 1996) was used. The method is based on radial migration of large DNA protein complexes in a high-gradient hydrodynamic field of a rotary viscometer (Taylor and Trotter 1974, Kryuchkov et al. 1995).

Radial migration of molecular complexes towards the rotating rotor causes anomalous changes of viscosity that can be registered by measuring the rotor rotation period as a function of time. The AVTDs were measured in the cell lysates at a shear rate of 5.6 s and a shear stress of 0.007 N/m^2 using an AVTD analyzer Archer-Aquarius (Russia). Maximum relative viscosity ($\eta_{\text{max-relative}} = T_{\text{max-exp}}/T_{\text{max-con}}$) was used to determine the magnetic field effect, where $T_{\text{max-exp}}$ is the maximum period of rotation, corresponding to maximum viscosity of the exposed samples, whereas $T_{\text{max-con}}$ is that corresponding to control sample under the same conditions.

The positive window at $9 \pm 0.5 \text{ Hz}$ and $16 \pm 2 \text{ Hz}$ observed with GE499 cells of *E. coli* are shown in Figure 22A. Only one window at $8.5 \pm 0.5 \text{ Hz}$ was observed with GE500 cells (Figure 22B): with a maximum effect exhibited at 70 min after the exposure. These four *E. coli* strains indicate that

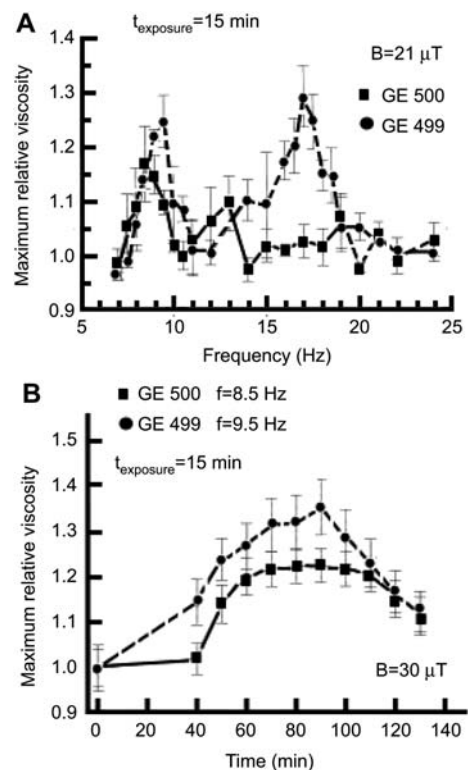


Figure 22 Frequency-dependent effects of ELF magnetic field on chromatin conformation in *Escherichia coli* cells. Experiments of Belyaev and Alipov (2001). The cells were lysed 70 min after the exposure.

electromagnetic resonances depend on the strain and genotype (Belyaev and Alipov 2001). Following these authors, the magnetic field effects on the cells can be coupled at different levels of the cellular organization addressing ions, radicals, macromolecules and organelles. The approach regarding resonance frequency, however, is based only on consideration of cyclotron frequencies, harmonics and subharmonics of biologically important ions. Only the models regarding the ions as targets predicted the values of resonance frequencies for extremely-low frequency (ELF) effects based on consideration of cyclotron frequencies, harmonics and subharmonics of biologically important ions (Lednev 1991, Liboff et al. 1991, Blanchard and Blackman 1994, Binhi 1997). According to Belyaev and Alipov (2001), the simulations for Ca^{2+} , Mg^{2+} and Zn^{2+} ions showed a remarkable consistency with experimental data and suggested that the same ions could be important targets for effects of static and alternating magnetic field in eukaryotic and prokaryotic cells.

In addition, the work of Del Re et al. (2006) should be mentioned where heat shock protein synthesis in *E. coli* under pulsed magnetic field of 50 Hz and 1 mT amplitude was studied. Two pairs of Helmholtz coils, 23 cm and 40 cm, in 23 cm diameter [with 40 (20+20)] turns was used to generate field pulses of sinusoidal and pulsed square (50% duty cycle) waveforms. It was found that bacteria exposed to a sinusoidal field exhibited significantly higher levels of DnaK and GroEL proteins when compared to the control sample. In comparison, similar results were obtained when bacterial cells were exposed to a heat shock after the magnetic field exposure. Because biological effects of MF seem critically dependent on the genetic background of the organisms tested (Belyaev and Alipov 2001, Fojt et al. 2004), as mentioned above, two different strains of *E. coli* (*TG1* and *DH5 α*) were used in the experiments. A procedure of heat shocks at 42°C, magnetic field exposure (1 mT, 50 Hz, 32°C) and again a heat shock (after the field exposure) formed the runs of the experimental tests. After the heat shock, an increase in DnaK and GroEL protein levels was observed, as expected, in both *TG1* (approximately 2 times) and *DH5 α* cells (approximately 5 times). The consequent exposure to the magnetic field led to reductions in the amounts of both proteins with regard to the control samples. The only exposure to magnetic fields resulted in reduction in the protein amounts in *TG1*. The same reduction was observed when a heat shock followed the pulsed magnetic exposure, too. In contrast, an increase of 1.5 times (with regard to the control volume) was observed when a sinusoidal field was applied and a thermal shock after that. The results with *DH5 α* were almost the same (qualitatively) as those obtained with *TG1*. It was concluded that the pulsed field has a relative effect with regard to the increase in the protein amount. In this context, it should be noted that pulsed fields mean high frequencies, due to the rapid increase (in time) of the pulse front. The same happens when the field is switched off (the back slope of the pulse). Therefore, the frequencies applied with the pulsed fields were much higher than those of the sinusoidal field of 50 Hz. Thus, higher frequencies, higher intensities of the eddy currents in the liquid medium can lead to:

- the consequence of an increase in the temperature, which is equivalent to a thermal shock. In addition, the electric (eddy) currents also kill the bacteria and destroy the morphology as it mentioned by Ji W et al. (2009),
- low intensity and high frequency do the same as high fields and low-frequency motions of shaking liquids (Ji W et al. 2009),
- a frequency of 50 Hz is enough high with regard to the shaking frequency of the samples under static fields to produce eddy currents and cause similar effects of reduction in protein amounts and damages in cell surfaces.

A particular study on the electromagnetic field effects on the protein molecular structure of *E. coli* and its pathogenesis was performed by Gaafar et al. (2008) in an electromagnetic field of 50 Hz and 2 mT strength. A solenoid consisting of

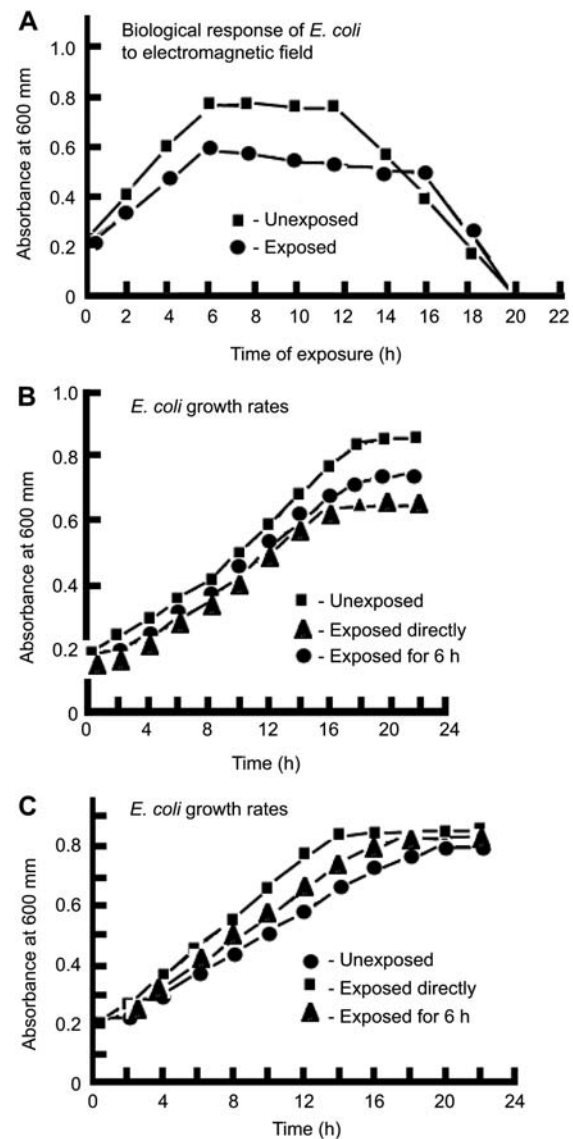


Figure 23 Biological response and growth rates of *Escherichia coli* in the experiments of Gaafar et al. (2008). Note that the data in (B) and (C) are from different experimental runs.

320 turns from electrically insulated 2 mm copper wire was used. The coil is mounted on a copper cylinder 2 mm thick, 40 cm diameter and 25 cm length which plays a role of an electromagnetic shield. Then, equal volumes were inoculated simultaneously and then the first volume was exposed to the magnetic field for 2 h; the second one for 4 h; the third one for 6 h and so on. The growth rates of all volumes (direct and late effect) were studied through measuring the absorbance of the viable cells after 2, 4 and 6 until 22 h. It was found that the exposure to the magnetic field decreases the cell numbers (Figure 23A). The growth rates for samples after 6 h exposures are shown in Figure 23B,C. More significant reduction in the growth rate was observed with the samples exposed for 6 h. Exposure time of 6 h was selected after an analysis of the preliminary data from samples where the cell numbers decreased from 10^8 (cell/ml) to 10^7 (cell/ml). Gaafar et al. (2008) attributed the inhibitory effects to electric charges induced by the time-varying magnetic field and the cytoplasm membrane, to reduction in their electric potentials and to consequent reduction in the cellular metabolic activity and biosynthesis.

The above commented results directed Gaafar et al. (2008) to dielectric measurements concerning the permittivity ϵ' , dielectric losses ϵ'' and the conductivity σ as a function of the applied frequency, of water soluble protein samples extracted from the cells by ammonium sulfate according to the method of Schleif (1981). Some of the results are shown in Figure 24.

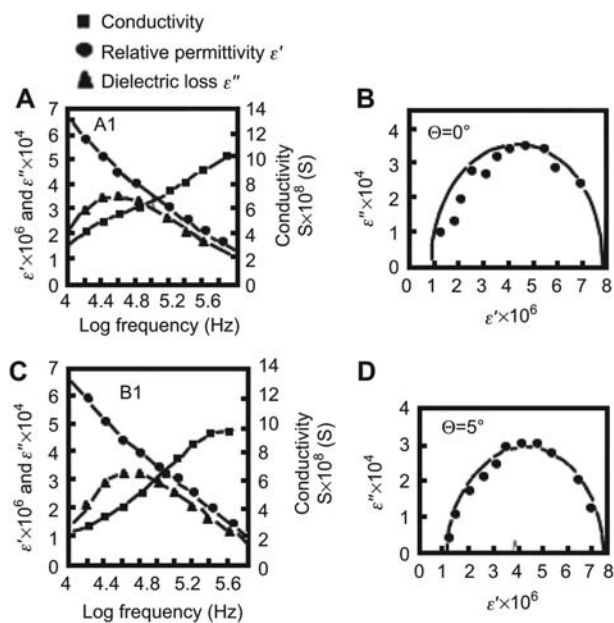


Figure 24 Dielectric measurements of *Escherichia coli* suspensions. Adapted from Gaafar et al. (2008). (A, B) Variation of the relative permittivity ϵ' , dielectric loss ϵ'' and conductivity σ as functions of the frequency. Samples A1 and B1 with direct field effect (exposure). (C, D) Cole-Cole plots, ϵ' versus ϵ'' corresponding to (A) and (B), respectively. Θ – angle of dielectric loss related to the Cole-Cole parameter $\epsilon' \propto \epsilon'' = \Theta$. Note that A1 and B1 are original codes (Gaafar et al. 2008) for cultures unexposed and exposed for 6 h to the electromagnetic field, respectively.

Based on the experiments of Gaafar et al. (2008) it follows that the relaxation time τ_{relax} decreases from 5.55 μs (unexposed organisms) to 3.88 μs and 3.54 μs for exposures of 6 h and 16 h, respectively. The decrease in τ_{relax} is an indicator for the decrease in the molecular weight of the protein molecules. Moreover, the Cole-Cole plots (Figure 24) have a parameter $\alpha_{\text{Cole}} \approx 0$ that indicates almost spherical forms of the protein (water-soluble) molecules of the unexposed cells. Exposures to 6 h and 16 h shift the Cole-Cole parameter to $\alpha_{\text{Cole}} \approx 0.05$ and $\alpha_{\text{Cole}} \approx 0.09$, respectively, thus revealing changes in the shapes of the protein molecule (from spherical to semi-circle forms) and alternation in the distribution of the charges over them.

According to Gaafar et al. (2008), the exposure fields have magnitudes significantly higher than the biomagnetic fields of the cells (generated by bioelectric pulses as a result of bending the cellular membranes). Hence, the external fields disturb the cell metabolic functions and lead either to death or augmentation in the cell division (Fadel et al. 2003). According to Gaafar et al. (2008), the disturbances in the cell division and the reduction of the number of cells after the exposure to magnetic fields lead to death or its suppression (Shin-Ichiro et al. 2002). However, the latter conclusion by Gaafar et al. (2008) combines two different approaches in the cell exposures under magnetic fields: high-intensity static fields and low-intensity time-varying fields, which, in fact, have distinct physical effects on the organisms, as discussed at large in this section of the review.

3.2.5.3. Brief comments on the magnetic field exposures of *E. coli* The results discussed to this point drew two main tendencies in the magnetic field treatment of *E. coli* with almost unified outcomes. To be precise, the high static magnetic fields suppress the death of the bacteria but to some extent it can alter their surfaces due to oxidative stresses. In any case, static high-intensity magnetic fields might be of some academic interest without encouraging results allowing implementation at a larger scale. The equipment required is huge, too expensive and represents a high-energy consuming power unit with a small working volume employed for cell treatment. Irrespective of these conclusions, the studies performed so far fill the gap in the knowledge about high-intensity static field effects on *E. coli*. In fact, the final results are almost the same as those obtained with other living organisms exposed to high-intensity but static magnetic fields, i.e., there is no effect on the cell proliferation.

In contrast to the static fields, the time-varying counterparts drastically affect the behavior of *E. coli* in two principle directions:

- The alternating magnetic fields (sinusoidal or pulsed) kill the bacteria or at least suppress their growth.
- There are positive resonant windows where the field tolerates the cell growth (Fojt et al. 2004). These windows depend on the bacterium genotype but are within the frequency range below 50 Hz.

The alternating field effects could be simply explained by the fact that there are interferences with the biomagnetic

fields at the level of the cells. The transport of ion across the membranes is also time-dependent (or time-varying) and the interference with the assisting fields having amplitudes and frequencies higher than those at micro-levels (cell membranes) alters the cell transport mechanism and metabolic activity. Moreover, to some extent, the alternating field can cause thermal shocks that also affect in a negative manner the bacteria viability. The common explanations in the studies analyzed about the mechanism of the field effect on the cell morphology and metabolic activity can be divided into two groups: a) field effects on the specific ion transport across the cell membranes and b) field effect on the molecular structure involving protein shape alternation and redistribution of the electric charges (Gaafar et al. 2008).

It is difficult to draw a more detailed picture but the above comments reveal that:

- The present state-of-the art concerning magnetic field effects on *E. coli* is somewhat stochastic and unsystematic. The choice of the field and its parameters as well as the strains used is rather voluntary and not well motivated. There is a deficiency of systematic studies in this direction.
- The processes at micro-level (cells) are rather complex to be simply explained. Most articles refer to one or two others where some suggestions about the mechanisms are developed, but their own analysis is missing.
- The external assistance by a magnetic field, if it is performed with not well-defined equipment and a tool, as mentioned in the previous comment, do not provide new data but, in contrary, generates additional gray noise contaminating the literature. To some extent, such data can be used in large reviews such as the present one, but as sole reports they make more noise than science.

3.2.6. Other processes exposed to magnetic fields

To complete the review of magnetically assisted proliferation studies, some other processes which are not so systematically performed as those with *S. cerevisiae* and *E. coli* but provide interesting data will be briefly reviewed. Not all can be encompassed but some representative studies will be referred to.

3.2.6.1. Static magnetic field effects on yeast and bacteria

The growth of *Bacillus subtilis* (aerobic bacterium) was investigated by Nakamura et al. (1997) by exposures in a superconducting magnetic system applying fields up to 7 T. These experiments show that the reduction in cell numbers exposed to the inhomogeneous field is significantly slower in the stationary phase. *B. subtilis* forms an endospore from vegetative cells when certain nutrients are depleted in the stationary phase. The apparent variation in total cell numbers also includes variations in the numbers of vegetative and spore cells. It was found that the number of spores in the magnetic field and in the reference (control) culture was practically unchanged. Moreover, the number of spores was relatively low with regard to the number of total cells. Nakamura et al. (1997) concluded that the magnetic field reduced

the dead rate of vegetative cells and inhibited the spore formation. The latter process begins at the onset of the stationary phase. In general, this study was performed in the manner of those with high static fields on *S. cerevisiae* and *E. coli*; the equipment and the research group was the same.

Utsunomya et al. (2003) tested the effects of an external magnetic field (0.13–0.3 T) on the production of porphyrin using *Rhodobacter sphaeroides* IF012203 under anaerobic-light conditions. The magnetic system used was an electro-magnet with an iron core; the light irradiated vessel (100 ml) was placed between the flat parallel poles. The vessel was divided in two compartments: N-pole compartment and S-pole compartment. Each compartment contained 50 ml of the culture broth. The experiments revealed that the cell growth was slightly suppressed and there were no differences in both compartments. Porphyrin production was enhanced at the N- and S-pole side compartments to 14.6 mg/l under 0.13 T exposure, whereas the control (non-exposed) production was only 6.7 mg/l. When the exposure at 0.3 T was performed, the porphyrin production was augmented to 38.8 mg/l.

The inhibition of bacteria growth in magnetic fields was studied by Piatti et al. (2002) in a static magnetic field 8 ± 2 mT for inhibition of *Serratia marcescens* growth. In addition, the same test considered a magnetic field effect on callus cell suspensions from *Hordeum vulgare* and *Rubus fruticosus*, but only the former exhibited reduced viability. When *S. marcescens* cells were grown in the absence and in the presence of a magnetic field, a decrease in the number of cells was observed at 24 and 48 h of incubation in comparison with control volume. Piatti et al. (2002) reported that from microscopic observations, it followed that many callus cells of *H. vulgare* were severely damaged.

Magnetic field effects on PHAs synthesis from different short-chain fatty acids by activated sludge was reported by Chen and Li (2008) in static magnetic fields (0–42 mT) in a SBR. To some extent, this study repeats the work of Yavuz and Celebi (2000). The results revealed forced specific production rates over the entire range of magnetic intensities applied. The authors claimed that their results are comparable to those of Yavuz and Celebi (2000) and Jung and Sofer (1997). The relevant explanations are based on the hypothesis of Blanchard and Blackman (1994).

Liu S et al. (2008) reported effects of static magnetic fields on enhanced anammox consortium activity for nitrogen removal from wastewaters. The experimental system resembles that of Yavuz and Celebi (2000) with permanent magnets mounted along the column (5 cm ID and 10 cm in height) wall. The magnetic field was varied in the range of 16.8–218.0 mT (by changing the distance between the poles of the permanent magnet). A window was found in the field intensity between 75 mT and 100 mT where the anaerobic ammonium oxidizing activities of anammox consortium reached a maximum. After an 80-day operation of the magnetic system anammox reactor, the maximum NH_4^+-N removal rate was 30% higher than that in the control experiment without the magnetic system. Moreover, the start-up time was shortened to 75% of that required for the control experiments. According to Liu S et al. (2008), the applied mag-

netic field affects the microbial composition of the anammox reactor by a highly enriched culture with *Planctomycetes*-related organisms: established by decreasing diversity of the denaturing gradient gel electrophoresis fingerprint.

The activities of fungi of Koji (*Aspergillus oryzae*) under strong magnetic fields (10 T) in a superconducting magnet were investigated in the production of Japanese Sake mash (Otabe et al. 2009). The main outcomes of this study are:

- the concentration of the glucose in the magnetic field is larger than that in the control field, and the concentration of the ethanol in the magnetic field is smaller than that in the control field;
- the number of the yeast cells in the magnetic field is suppressed with regard to that in the control experiments.

According to Otabe et al. (2009), the results suggest that the activity of the yeast cells is suppressed by the strong fields and the glucose is not sufficiently converted to the ethanol (resulting in sweet taste). Furthermore, they decided that if the fermentation rate is controlled by the magnetic field, new types of taste could be created in new circumstances with the magnetic fields. For example, much slower fermentation could be achieved at low temperature and strong magnetic field.

The time dependence of the number density of the yeast cells in the suspension (counted by standard plate count technique) was analyzed in terms of the Malthus equation as given by

$$\rho_{\text{yeast}} = \rho_{0\text{-yeast}} \exp(k_{\text{prof}} t) \quad (3)$$

where $\rho_{0\text{-yeast}}$ is the initial number density of the yeast cells at initial time $t=0$; k_{prof} is the growth (proliferation) coefficient.

In general, the values k_{prof} in magnetic fields are lower than those obtained for the control non-exposed volumes. It was observed that the differences in the growth coefficient became large as the field intensity was increased and become almost constant beyond fields of 5 T. The growth coefficient variation with field and temperature was expressed in an Arrhenius-like relationship (Otabe et al. 2009), namely:

$$k_{\text{prof}(B)} = k_{\text{prof}(B=0)} \exp\left(\frac{m^*}{k_B T} B\right) \quad (4)$$

where k_B is the Boltzmann constant and m^* is the magnetic growth activation moment which represents the sensitivity to magnetic field (Neumann 2000). Large values of m^* mean that the growth activation is sensitive to the magnetic field.

The study of Otabe et al. (2009) is typical as a proliferation study, but gives an example of how data have to be correlated with the intention of extraction of more logical and well-organized data.

3.2.6.2. Time-varying magnetic field effects on yeast and bacteria Lei and Berg (1998) found out that positive electromagnetic windows exist for proliferation of *Corynebacterium glutamicum* at approximately 15 Hz and 4.9 mT.

The effect was observed in the exponential phase starting after 4 h of the inoculation onset. The maximum effect was observed after 2.5 h since the beginning of the exponential phase. This study does not differ in its style and results (only the biological object was changed) from others published by H. Berg (see Mehedintu and Berg 1997, for example).

Looking at the variety of results published and commented in this review, it has to be asked: What is the reason for the field effect in both positive and negative directions? Too many explanations provided in the articles coincide with magnetic field effect on charges concerning ion transport through the cell membranes and effects on the intracellular proteins, thus resulting in altered metabolic activity of the exposed cells. In this context, the study of Marron et al. (1988) is referred to, which was particularly oriented to field (electric and magnetic) effects on cells under 60 Hz alternation frequency, i.e., a case too close to all encompassed here. *Physarum polycephalum* amoebae were exposed for 24 h to either or both 60 Hz sinusoidal electric and magnetic fields, applied at levels of 0.1 mT and 1.0 V/m, respectively. The simultaneous application of both fields was performed in a horizontal plane (of the sample medium), in phase, but orthogonal to each other. A chromatographic technique called two-phase aqueous polymer thin layer countercurrent distribution (TLCCD) (Walter et al. 1985, Albertsson 1986) was used to study the effects on the cells. The results lead to the conclusion that both the electric and the magnetic fields alter the cell surface, independently, and in different ways:

- The electric field exposure produces an increase in the net negative surface charge of the amoebae.
- The magnetic field exposures result in a decrease in the hydrophobic character of the amoebae.

Marron et al. (1988) correctly mentioned that the pulsed magnetic fields produced concomitant electric fields. Consequently, cells exposed to pulsed magnetic fields displayed a response to TLCCD similar to the exhibited ones when both fields are applied. This comment by Marron et al. (1988) relates to the MLD concept conceived at the beginning of the present section of the review (see the section devoted to *S. cerevisiae*).

Another important comment by Marron et al. (1988) is that the exposure required to obtain some effects on the cells should be long enough. In these experiments, exposures of 24 h led to detectable changes in the cell membrane surfaces, whereas exposures of 18 h resulted in weak changes. Longer exposure did not alter the results obtained during 24 h. Based on this particular study and on the basis of other results discussed here, it is possible to state that the exposure time with detectable effects strongly depends on the type of the cells, bacteria strains, their genotypes and the field intensity, and also frequency.

At present, it is rather difficult to create a general map, because the variety of living microorganisms affected by external magnetic (and electric) fields is large enough. The information available in the literature in many cases is insufficient, not well supported by experimental data concerning

both experimental conditions and results. Hence, more detailed and systematic studies are required to find common elements and reliable data allowing correlations prior to creating general conclusions. Despite this “obscure atmosphere”, the analyzed results allow, to some extent, to see what could happen and would be expected if a certain living microorganism of interest is forced by external magnetic fields to die or to augment its metabolic activity. Then, experiments, correctly arranged and systematically performed, have to be carried out.

3.2.6.3. Time-varying magnetic field effects on enzyme activity

At the end of this section we will briefly comment on the time-varying field effects on enzyme activity. Significantly high-frequency fields, with regard to all discussed so far, were used by Blank and Soo (1998a) to increase the activity of cytochrome oxidase. The magnetic field component was low at 10 μ T. Cytochrome oxidase was prepared from livers of Sprague-Dawley rats (Blank and Soo 1998b) and enzyme activity was determined by the method of Smith (1955). Changes in concentration of oxidized cytochrome in time was used to calculate the oxidation rate constants for both exposed and control samples. An increase was found in the reaction-rate constant which varies with the frequency of the magnetic field in the range 10–2500 Hz. Moreover, it exhibits a maximum in the window 500–1000 Hz. The maximum is in the range of observed frequencies of the electron transfer reaction, but it is above the normal frequency range of 50–200 Hz for the cytochrome oxidase reaction in mitochondria.

The simplest explanation of Blank and Soo (1998a) concerning the close link between effects of electric and magnetic fields and enzyme activity is based on the mobile charge interaction model. The model suggests magnetic field interaction with moving charges, and hence the basal enzyme activity is the result of the field interaction with charge movements during enzyme function. Blank and Soo (1998a) also referred to probable effects of Lorentz forces that clearly indicate the complete misunderstanding in the interpretation about the observed phenomena, because the field was somewhat weak to create any effective ponderomotive forces.

We finish the overview of other processes boosted or decelerated by magnetic field exposures. It demonstrated at a glance what happens among the astonishing plethora of articles dealing with field effects on cell and bacteria. Most of them are typical case-study reports with only data and missing interpretations. In this context, the “digestion” and interpretations of the information are tasks needing enormous efforts and time, but sometime missions impossible due to insufficient information. The study of Neumann (2000) draws an idea of systematization of the information, as was done particularly by Otabe et al. (2009). However, this needs time and features future studies and analyses.

3.3. Reactors with recirculation and external culture magnetization

In contrast to the common approach concerning the working volume for proliferation or fermentation experiments to be

entirely encircled by the magnetic system, as in the dominating studies, discussed in this review, a small group of studies suggesting an alternative approach exists (Alvarez et al. 2006, Justo et al. 2006, Li ZY et al. 2007). The concept of entirely surrounded by a magnetic system working volume has some disadvantages concerning the limited volume and an increase in the energy losses with increase in the equipment scale. The magnetically encircled reactors with free cells are good enough for laboratory-scale experiments: proliferation and fundamental studies. The concept of magnetically encircled reactor, however, works rather well at large scales when magnetic supports are used as will be shown later in this review.

The alternative approach to the encircled reactors is the so-called intermittent, periodic magnetization (Chacon et al. 1996, Justo et al. 2006, 2007, Li ZY et al. 2007, Perez et al. 2007). In general, the concept means a closed loop including the reactors and a small chamber for magnetic exposure. The circulation of the culture medium (Figure 25) is driven by a pump (Chacon et al. 1996, Justo et al. 2006, 2007, Perez et al. 2007) or due to gravity (Raja Rao et al. 1997, Li ZY et al. 2007). It passes through the magnetization chamber and enters the reactor again. The residence time for magnetization depends on the intensity of circulation, the cross-section of the exposure chamber, and its length. The studies of Chacon et al. (1996), Justo et al. (2006, 2007) and Perez et al. (2007) deal with almost equal experimental equipments (Figure 25A,B) with small differences in the type of the magnetization equipment.

3.3.1. Forced circulation loop of a batch submerged fermentation process

E. coli growth in these experiments (Justo et al. 2006) will be analyzed, because with the background of similar studies performed in encircled reactors the advantages and the disadvantages of the periodic magnetization can be seen. The strain of *E. coli* used by Justo et al. (2006) was from the American Type Culture Collection (ATCC 25922) inoculated in 500-ml Erlenmeyer flasks containing 300 ml of sterile medium. The process (submerged fermentation) was started with 10% (v/v) inoculum of *E. coli* (approximately 106 cells/ml) in a Marubishi stirred glass fermentor of 1.2 l working volume at pH=7.0, 25°C and 350 rpm of the stirrer. The magnetic system (Chacon et al. 1996) used in this study created a static magnetic field in the range 10–100 mT (300 Hz). The magnetization chamber was a stainless steel double U-tube of 5 mm ID diameter and 20 cm of length; hence, there is a double pass through the solenoid. The circulation volumetric rate was 19.625 ml/s (1 m/s superficial velocity in the U-tube). Under these conditions the exposure time was approximately 40 ms (0.04 m exposure length with a velocity of 1 m/s). With recirculation velocity, the entire working culture of 1.2 l can be completely recirculated through the loop for approximately 61.146 s; hence, once per minute, the cells pass through the exposure chamber or the entire culture (1.2 l) passes approximately 588 times per 1 h through the exposure chamber. The short exposure time of 40 ms resembles a pulse of 25 Hz pulsed

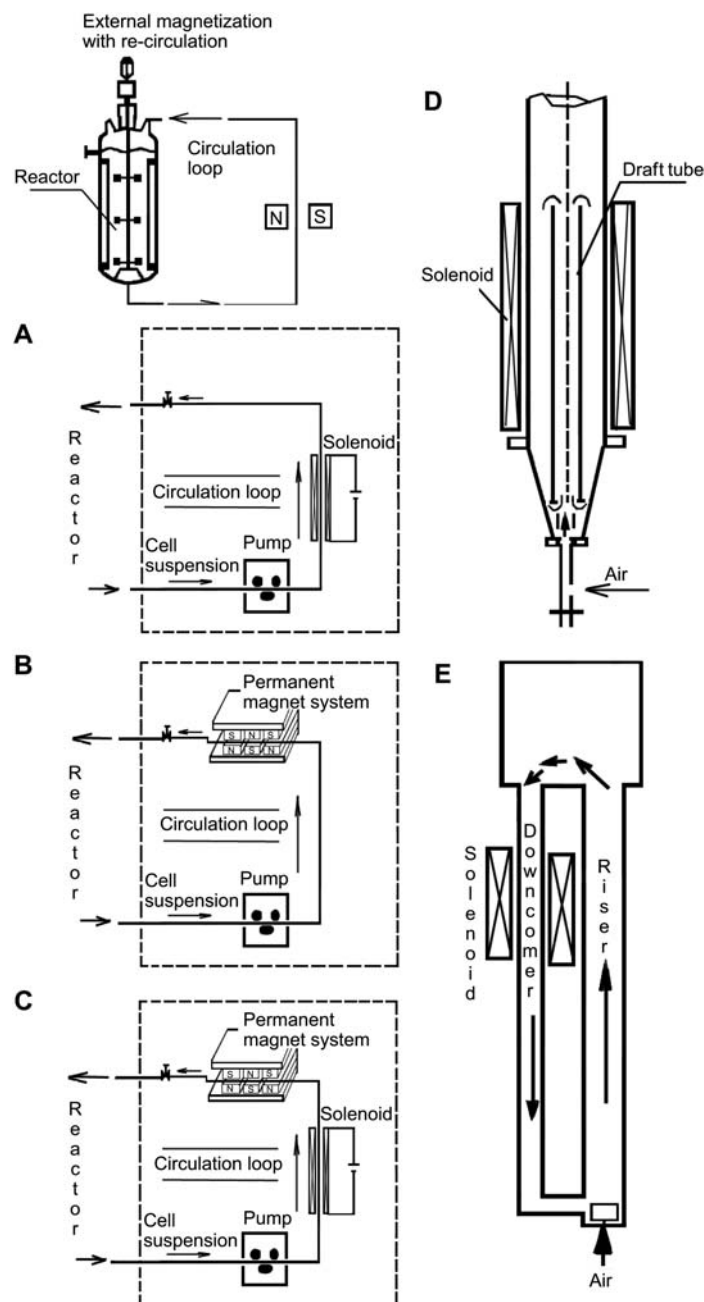


Figure 25 Fermentation with recirculation and external magnetization. (Top) Basic principle with a pump driven loop circulation. (A) Magnetization loop of Justo et al. (2006, 2007). Growth of *Escherichia coli*. (B) Magnetization loop of Alvarez et al. (2006). Nisin production. (C) Magnetization loop of Perez et al. (2007). Ethanol production by *Saccharomyces cerevisiae*. (D) Internal recirculation and magnetization in a draft-tube airlift (Raja Rao et al. 1997). (E) External circulation and magnetization in an external-loop airlift (Li ZY et al. 2007). Batch cultivation of *Spirulina platensis*.

static field, which, to some extent, is in the middle of other ranges used in the studies reviewed here.

The effects of the independent variables such as electromagnetic induction and the exposure time were evaluated on the growth of *E. coli*, using a Box-Wilson central composite design. Viability was measured by the conventional technique of total viable bacterial counts determined by the CFUs. The number of colonies and the biomass/substrate yield ($Y_{x/s}$ – the yield coefficient of the Monod model [Eq.

(2)] were affected by the field exposure when compared to the control fermentation run. Higher values were achieved for the microbial biomass and for the $Y_{x/s}$ at the largest exposure time (in fact, the total time of recirculation through the magnetization chamber): between 6.5 and 12 h. In the latter case (12 h) the field was approximately 55 mT. As the microbial cells were fixed in the culture medium, several visible and isolated colonies were formed. However, it was not possible to establish a direct relationship between the number

of formed colonies and the number of cells (Chacon et al. 1996) that avoided recording lethal effects of *E. coli*. In this context, the authors mentioned that viability was almost unaffected by the magnetic field, but the highest one was observed in the control (non-magnetized culture) experiment. By contrast, the yield coefficient $Y_{x/s}$ varied from 6.5% to 49.2% – the best result corresponds to a field of 55 mT for 12 h circulation. In all other runs (different field intensities), the authors claim inhibitory effects of the magnetic field (based on low values of $Y_{x/s}$). It was estimated experimentally that the optimal process performance was of 6.5 h circulation through the exposure chamber; this simply means approximately 3827 complete circulations of the entire culture medium (1.2 l).

Based on the experiments of Justo et al. (2006) it is difficult to establish links to the proliferation studies analyzed in this section. In general, there was a window in the time of exposure as a cumulative measure of the magnetic action on the culture medium. This, to some extent, might be considered as a measure of the total energy transferred from the field to the cells under the specific circumstances of the experiments carried out. Moreover, it is interesting to obtain information if the mixing through the loop added to that created by the stirrer influences the process or the only cause is the magnetization during the cell growth. In any case, any additional mechanical mixing will boost the process to some extent. Simple calculations reveal that with a velocity of 1 m/s and a tube diameter of 5 mm ID, the approximate Reynolds number in the magnetization chamber (calculated with the properties of water because physical properties of the culture medium are missing) is approximately 5000. The latter means an intermediate mixing of the culture and increase in the shear stresses in the culture medium. With a 1 m/s and a tube diameter of 5 mm ID the velocity gradient from the tube centerline to the wall is approximately 400 s that yields (with the dynamic viscosity of the water) shear stresses of approximately 4 N/m². These data will be recalled for analysis of the overall performance of the bioreactors with recirculation.

Similar experiments but for nisin production by *Lactococcus lactis* subsp. *lactis* using cheese whey permeate were performed by Alvarez et al. (2006). The diameter of the tube of the recirculation loop was the same (5 mm ID) but the magnetizing system was replaced by a tooth-shaped permanent magnet system. According to these authors, such a system with a periodically altered pole of permanent magnet prisms creates a space varying field with longitudinal zones with strong and weak fields that would work like magnetic pulses (the main concept of the authors). In fact, the magnetic field cannot be isolated locally and this system does not work as was imagined: the field is inhomogeneous along the exposure chamber and there are no magnetic pulses. According to Alvarez et al. (2006), the field in the gap of the magnetization chamber was adjusted at three levels: 5 mT, 12.5 mT and 20 mT. An additional element, in the recirculation loop, with regard to the experiments with *E. coli*, is the variable circulation flow rate of 0.85 m/s to 1.5 m/s, which was equivalent to a magnetization with a frequency

of approximately 25 Hz. However, the recycling velocity was kept at 0.85 m/s for all fermentation runs. The analysis of the experiments of Justo et al. (2006) showed that the frequency of magnetization in those experiments was approximately 25 Hz. All other facilities used are the same as those in Justo et al. (2006).

The experiments revealed that there were positive windows in the biomass production (Figure 26A) and the product yield coefficient $Y_{p/s}$ for nisin (Figure 26A,B). ANOVA was used to establish the optimal conditions. The final results reveal that:

- Under a magnetic field of 20 mT, the substrate consumption and the cellular growth at the first 4 h of fermentation were twice higher than those of the control medium.
- The fact that the nisin production started at 8 h of fermentation, when the biomass production reaches the stationary phase, indicates that the nisin production is not related to the cellular growth.
- It was not necessary to maintain the cells recycling through the magnetic field during the entire fermentation course. Only 4 h of exposure was enough to obtain the

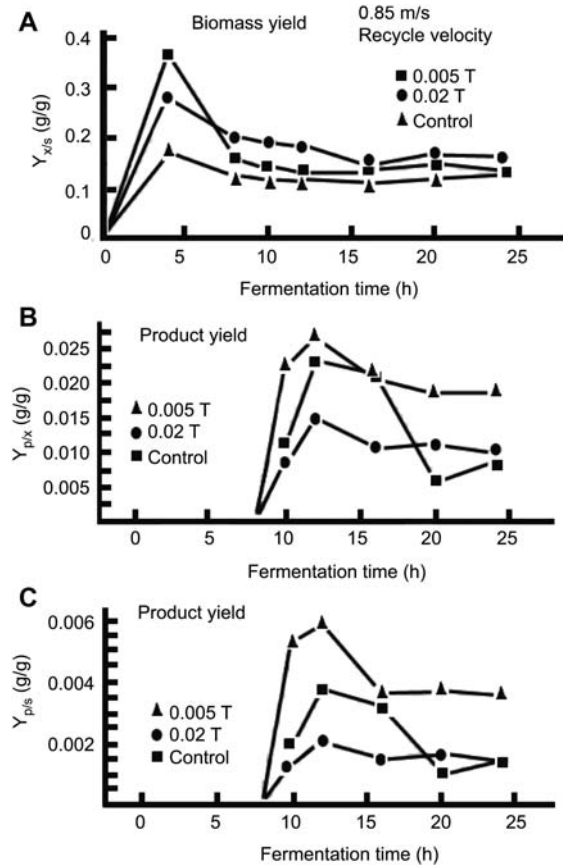


Figure 26 (A–C) Growth kinetics and nisin production by *Lactococcus lactis* with application of magnetic fields aiming to control the experiment during 24 h of fermentation. Adapted from Alvarez et al. (2006).

highest nisin production in comparison with the control assay.

- d. The outcome of the models created by ANOVA shows that the magnetic field effect has an inhibitory effect on the nisin production.
- e. With a fixed recirculation flow rate, it was not possible to establish the effect of this operating variable.

Furthermore, the strain of *S. cerevisiae* was tested for ethanol production in almost the same experimental set-up used for nisin production (Alvarez et al. 2006). Only an additional magnetic field source was added in the circulation loop (Figure 25C). The operating parameters are the same as those in the study of Alvarez et al. (2006). The experiments were designed using ANOVA and the analysis of experimental data led to the conclusion that cell suspension could involve an electric field or magnetic and electric fields induced in the conducting culture and alter the metabolic activity. This is an explanation with weak links to the MLD concept developed earlier in this section.

The ethanol production varied with the recycle velocity showing two positive responses for 0.9 m/s and 1.2 m/s (figure 4 in the original work). The authors attributed this effect to the frequency of magnetization. The yield of ethanol at 0.6 m/s and 1.2 m/s was almost the same ≈ 4.2 (g/l/h), but lower than that observed at 0.9 m/s and 1.2 m/s (≈ 5.75 g/l/h). The difference in 12% was considered as “a window effect” with regard to the contribution of the circulation velocity to the total process performance. The latter was also

attributed to the field effect, i.e., frequency of magnetization. The experiments will be discussed later in this section due to combined effects of high shear stresses and macro/micro mixing caused by the recirculation.

3.3.2. External-loop airlift with magnetization A draft-tube airlift entirely exposed to a magnetic field (Figure 25D – no detailed data exist in the paper) was applied by Raja Rao et al. (1997) for degradation of glucose-urea and phenol wastewaters by a mixed culture of microorganisms. The culture was adapted to degrade phenol gradually (day by day). The major results are:

- a. the magnetic field increases the rate of microbial growth (Figure 27A) in all four stages of living cells in a batch process,
- b. the phenol degradation at 110 Oe is faster than in the absence of a field (Figure 27B). The authors attributed the fact to an increased population density of the microbes. Particular experiments performed indicated that the field intensity of 110 Oe increases the mixed liquor suspended solids from 2.4 g/l (zero field intensity) to 3.4 g/l.

An external-loop airlift photo-bioreactor (3.5 l working volume) was employed by Li ZY et al. (2007) for cultivation of *S. platensis* (Figure 25D). The magnetization (up to 550 mT) was performed by a solenoid through which the airlift downcomer passes. The growth curves of *S. platensis* under various field intensities in the solenoid are shown in

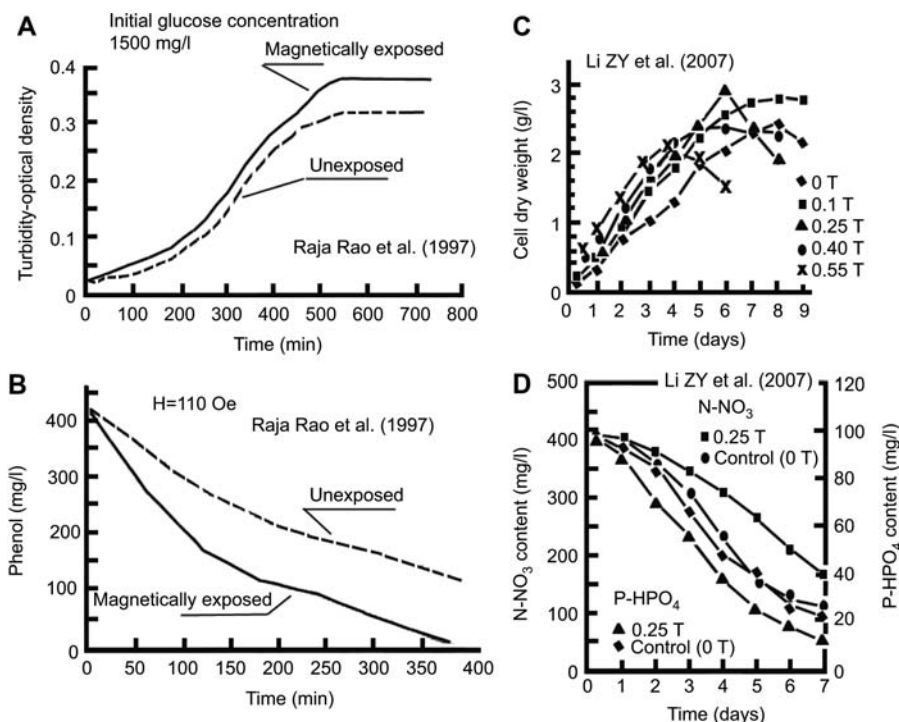


Figure 27 Growth and kinetics in gas-driven circulation reactors with magnetizations. (A) Raja Rao et al. (1997): field effect on the microbial growth. (B) Raja Rao et al. (1997): variation in phenol degradation-suspended growth system. (C) Li ZY et al. (2007): growth curves of *Spirulina platensis*. (D) Li ZY et al. (2007): N-NO₃ and P-HPO₄ in both the magnetically exposed and the control system.

Figure 27C. The optimal, i.e., maximum growth was obtained at 0.25 mT (Figure 27D). The air flow was fixed to 20 h space velocity only to circulate the liquid through the airlift loop. Again, a statistically designed experiment was used to find the optimal conditions. This is a study with a good concept but with rather obscure results for many reasons, including:

1. The liquid circulation in airlifts depends on the gas flow rate through the riser. However, neither the gas flow rate was changed nor it is known from this article because it is presented as a space velocity $\left(\frac{Q_{air}(m^3/h)}{V_{riser}(m^3)} \right)$.
2. It is impossible to detect if the magnetization boosted the *Spirulina* growth or not. The field intensity range corresponds to that used by Hirano et al. (1998) in a proliferation study. The optimal intensity of approximately 250 mT and the inhibition in the cell growth at higher fields also confirm the results of Hirano et al. (1998).
3. The only positive moment is the use of an external-loop airlift allowing further development if the experiments will be carried out professionally.

3.3.3. Comments on the bioreactors with external loops and intermittent magnetizations (Questions and answers related to the cell survivability in bioreactors) The reactors with recirculation and external magnetization seriously set up some questions, including:

- A. Is the external recirculation the suitable engineering approach to boost magnetically bioprocesses conventionally performed in large vessels?
- B. Does the recirculation affect the cell (bacteria) viability, growth and metabolic activity?
- C. What is the contribution of the recirculation to the overall mixing of the cultured medium and its impact on the cells and consequently on the process performance?

The questions cannot be answered directly from the information in the revised articles (Alvarez et al. 2006, Justo et al. 2006, 2007, Perez et al. 2007) but we will attempt to do that by obtaining information from studies not directly related to magnetic field effects on living microorganisms.

- First of all, question “A” addresses scale-up effects and adaptation of conventional bioreactors to process intensification via external magnetizations. The circulation loop driven by a pump could be single or multiplied (i.e., many circulation loops driven by pumps and many external magnets). The first option (single loop) should be scaled as the fermentor size will rise; that is, the conditions at the laboratory scale could be fulfilled as the scale of the circulation loop grows. By contrast, the multiplication loop allows the circulation conditions at a laboratory scale to be the same at the large scale, but this approach does not meet many requirements concerning pump maintenance, loop sterilizations, circulation control, many magnets at work, etc. A more reasonable approach is that suggested by Li et al. (2007) with external mag-

netization in the airlift downcomer rather than the entire reactor to be magnetized (Raja Rao et al. 1997). This concept, however, should be developed by increasing the reactor scale.

- Question “B” refers to any effects of mechanical and hydrodynamic origin that could have an impact on the cell viability and growth during the recirculation. Intensive motions in culture media cause extracellular shear stresses affecting the living microorganisms. Only two of them most frequently used in magnetization studies, *E. coli* and *S. cerevisiae*, will be commented on.

E. coli (MG1655) in a low-intensity extracellular shear stress environment was investigated by Tucker et al. (2007) and subjected to transcriptional and physiological analysis. A high aspect rotating vessel with aerobic rich LB and minimal (MOPS-glucose) media was used. The study encompassed physiology, stress resistance and transcriptional analysis in both rich and minimal medium. The results reveal no effects of the shear stress on the lag phase of the growth processes carried out in the rich and minimal media. Culture growth kinetics were used to determine if low-shear stresses (LSSs) altered *E. coli* MG1655 physiology in a growth dependent manner. *E. coli* MG1655 responds to LSSs at the level of transcriptional regulation. The cellular physiology was only affected in rich LB medium where a decreased exponential growth rate compared to the control was observed. In minimal MOPS medium, chemotactic and flagellar genes as well as genes involved in the *E. coli* acid tolerance response were upregulated in the LSS environment. Tucker et al. (2007) suggested that LSS upregulation of flagellar and chemotactic genes in minimal medium is related to a cellular requirement for relocation away from zones of local nutrient depletion and the tendency to occur in the low mixing environment (i.e., in a calm environment with low mechanical stresses). In rich medium, the majority of the *E. coli* MG1655 genes that respond to the LSS environment were downregulated, presented in the cytoplasm, and involved in translation.

Allen CA et al. (2008) also performed LSS experiments with *E. coli*. It was found that an adherent-invasive *E. coli* O83:H1 exhibits an enhanced thermal stress resistance. In contrast, no significant differences were observed for osmotic and acid stresses. The study concluded that growth under LSS can alter bacterial resistance to different stresses but these changes are not universal and vary between strains.

The large scale experiments of Hewitt et al. (2000) with *E. coli* fermentation revealed that the percentage of viable biomass increases with a decreasing quality of mixing. They suggested that in a heterogeneously mixed bioreactor with continually fluctuating microenvironments, many interlinked regulatory stress-response pathways of the cells can be induced. Under worse conditions (Andersson et al. 1996), there are rapid increases in the concentration of the intracellular signaling molecules (ppGpp and cAMP) than in the

transcription and expression of several stress proteins. This phenomenon is known as the “stringent response” (Andersson et al. 1996, Teich et al. 1999) and the consequences include: the inhibition of DNA replication initiation, stable rRNA synthesis, and hence enzyme production. In addition, glycolytic activity, DNA metabolism and the synthesis of structural components are greatly reduced. The latter allows the “deep sleeping” cells to survive periods of nutrient substrate limitation (Hewitt et al. 2000).

Furthermore, Hewitt et al. (2000) reported a very high percentage of dead cells ($\sim 20\%$) at the end of a well-mixed, laboratory scale fermentation. They suggested that the dead cells had inabilities to survive in the conditions of a homogeneous microenvironment: this happens at the high cell densities achievable in fed-batch culture with prolonged and fully sustained (i.e., spatially and temporally) nutrient limitation in the well-mixed case (Hewitt et al. 2000).

S. cerevisiae (var. Vinal) under strong stirred fermentation was investigated by Converti et al. (1996). The stirrer speed was established at 600 rpm, whereas the fermentor operated normally at 300 rpm. The threshold of 450 rpm was established as a point where the external diffusion resistance to the medium was reduced and the cell metabolism became of greater importance in the mass transfer process. Moreover, because the carbohydrate fermentation by yeast is an anaerobic process, the intensive mixing causes surface aeration that leads to predominant growth of the cells to the intolerance of ethanol yield near the vortices and bubbles in the medium. This was attributed to the inhibition effect of the mechanical stresses (Converti et al. 1993). The product yield falls with an increase in the rotational speed beyond 500 rpm. Converti et al. (1993) suggested an increased amount of dead cells due to the excessive mixing. They also suggested metabolic disorders due to short-time mechanical damages of the cell surfaces. The cell viability was tested under isotonic conditions and as a result the number of living cells dropped approximately 2 times when the rotational speed was increased from 300 rpm to 650 rpm after 15 min exposure, and to 20% of the initial cell number when the exposure lasted 25 min (figure 5 in Converti et al. 1996). In terms of the Monod kinetic model the results of Converti et al. mean low product yield (figure 7 in Converti et al. 1996) and a fall in the maximum specific productivity with an increase in the exposure time to shear stresses. In contrast, the study of Boswell et al. (2003) does not report any effects of the mechanical stresses on the viability and growth of *S. cerevisiae* cells. However, these authors particularly mentioned that their experiments were not direct mimics of the batch, anaerobic conditions found in brewing. In the context of the discussion, the study of Chamsartra et al. (2005) with *Corynebacterium glutamicum* fermentation should be mentioned, where also no effects of mixing were detected in the large fermentation vessel.

Analyses and overviews on viability and cell damages of living organisms were performed by several reviews (Lara et al. 2006, Trujillo-Roldán and Valdez-Cruz 2006). The viewpoints are not unique, vary from organism to organism and survivability depends on both the organism genotype and

the mechanical conditions in the fermentation medium. Commonly, more severe conditions are created in a small fermentor rather than in large ones due to excessive fluid mixing.

Following this brief overview of the mechanical stress effect on living cells and bacteria, the conditions created in the bioreactors with external magnetization and external circulations loop should be mentioned. The concept to create pulsed magnetization by rapid passing of liquid through gaps of permanent magnets and solenoids, in fact, led to excessive mechanical stresses in the cell suspensions, even though the stirrer speed inside the reactor was at 300 rpm in all experiments. This hypothesis attempts to answer the question: Why did, in all those experiments the magnetization, in fact, the recirculation through the loop, lead to results lower than those observed in the control fermentation run? In general, it refers to question “C”, whereas the above comments on shear stress effects on cells answers question “B”. The only answer taken from the published data exists in the study of Alvarez et al. (2006) concerning production of nisin by *Lactococcus lactis* subsp. *lactis* (see table 1 in the original work). Two control experiments performed, with and without circulation, in the absence of a magnetic field resulted in:

- approximately 50% reduction in specific production yield coefficient $Y_{p/s}$ (nisin) when the loop was used;
- 3 times lower in the specific nisin yield per mass of formed biomass $Y_{p/x}$ due to recirculation;
- no remarkable changes in the consumed substrate (approximately 5% augmented consumption in the recycle) and the growth coefficient $Y_{x/s}$ (from 0.110 g/g without recycle to 0.120 g/g with recirculation);
- however, when the field was applied to the recirculation loop, the yield was $Y_{p/x} = 86.53 \text{ (g/g)} \times 10^{-2}$ (nisin yield per formed biomass) with regard to $Y_{p/x} = 18.23 \text{ (g/g)} \times 10^{-2}$ in the control fermentation with recirculation. Hence, the magnetization boosted the process, but the problem with the mechanical stress effect remained.

The experiment of Alvarez et al. (2006) is a good example of discrimination of the mechanical and magnetic effects. However, only one diameter of the circulation loop was used. In this context, future experiments should at first test the process with different loops (variations in the tube diameter). This suggestion envisages experiments equivalent to the studies of Converti et al. (1993, 1996) where mechanical stresses were produced by a stirrer.

3.4. Comments on free-cell magnetically assisted bioreactors

The present section reviewed many studies on magnetic field effects on living organisms. Only those relevant to biotechnology and with potential applications were reviewed. However, the explanation of the results needs deeper analysis and well-performed studies which have no direct associations with biotechnology but help us to understand the basic mechanisms of magnetic field effects. Some outcomes, both positive and negative, are briefly outlined next.

A) Positive outcomes

- There are positive windows in the cell proliferation at low-frequency and low-intensity field exposures.
- High static fields suppress the cell growth and proliferation, mainly *E. coli*.
- The fermentation runs with *S. cerevisiae* in batch reactors with mixing (experiment of Motta et al. 2001, 2004) and those of Perez et al. (2007) strongly indicate that there are also positive windows when static and pulsed fields are applied, respectively.

B) Drawbacks

- Most proliferation studies do not provide answers or suggest ideas how the results can be developed towards large scale.
- With regard to the previous comment, when fermentations are performed, the articles only mention some proliferation studies but do not go into depth of the problems trying to explain the macro results obtained. This simply means that there is a gap between both types of studies and missing interrelations between the results developed.
- Most of the published reports provide insufficient information and, to some extent, such articles only mark the area jumping from one living organism to another, repeating the research methodology: the outcome is an augmented proliferation of articles which do not provide either science or reliable data.
- The concept to use statistically designed experiments envisages reduction in the amount of work but it only masks the situation at issue, and at the end the information collected is insufficient for analysis. Statistically designed experiments are power tools when the systems are complex but well-known as mechanisms of interaction, and only the optimal conditions and the contribution of the most important factors have to be established. In the case of a magnetically assisted bioreactor the behavior of the systems are generally unknown, and the common approach is: let us see what it would be if a magnetic field is applied, followed by improper design of magnetic systems and bioreactors.
- The living microorganisms in bioreactors are exposed to many rough conditions because, in fact, the conditions do not replicate exactly their natural environments. Mixing, overgassing, changing pH, strong shear stresses, are common in laboratory and industrial fermentors worldwide.
- Magnetic field exposures contribute to stresses imposed on the cells and bacteria changing their natural habit of life. Hence, magnetically assisted reactors create too many stresses to the living microorganism and the route to the best reactor performance passes through careful preliminary analyses, correct design and systematically carried out experiments.

Some targets

In the context of the previous comments, the correct design of magnetically assisted reactors is of primary importance.

The mechanistic combination of a traditional fermentor and a magnetic system commonly designed improperly leads to discouraging results. This comment addresses the common practice in the reviewed studies. The proper design should start with proliferation studies, followed by analysis (and experiments if necessary) of the mechanical stresses in the absence of magnetic fields. The effect of shear stresses has to be minimized prior to the exposure to magnetic fields and this directly addresses the proper reactor design. Then, systematic studies concerning all aspects of the reactor and culture performance have to be carried out.

The mentioned outcome and the targets drawn just figure what has to be done and what has to be avoided. The development of these concepts needs interdisciplinary knowledge at the edge of fluid mechanics, cell biology, reactor design and magnetism (at micro-level and an engineering design, too).

Prior to start your own research on a magnetically assisted free-cell bioreactor and reading articles on macroscopic fermentation experiments, some important sources of information are recommended, including: Hong (1995), Mittenzwey et al. (1996), Berg (1999), Ishisaka et al. (2000), Dini and Abbro (2005), Morelli et al. (2005), Pazur et al. (2007), Blank and Goodman (2009), and Hunt et al. (2009).

4. Magnetic supports**4.1. Why magnetic beads?**

The presence of magnetic biocarriers in the literature is astonishing and they are normally acceptable as materials that have to be developed for certain applications. However, developing the analysis of MAF and especially of bioapplications, the main targets in the development of magnetic bio-supports have to be defined in an explicit manner.

Controlling the particle motion is the primary objective, well-known from the development of MAF. The basic magnetic materials such as iron, nickel, cobalt and magnetite do not work well as supports for cell and microorganism immobilization that raises the problem for creation of biocompatible magnetic composite materials. Then, all well-known features of the MAF such as controlled motion, working under fixed bed conditions at high fluid velocity, reduced diffusion mass transfer resistance, etc. can be utilized at full scale.

Separation of cells or enzymes from the culture medium is a mandatory step in the downstream processing. The traditional thermal shock, filtration and disintegration techniques create stresses in the liquids containing the metabolic products, which are the final targets of the upstream processes. The thermal and mechanical stresses reduce the content and the quality of useful metabolic products. The magnetic supports allow easily removing the immobilized cells or enzyme to avoid the negative effects of the traditional downstream processing.

Separation focusing is an advantage of magnetic biocarriers in the downstream processing. This simply means making magnetic particles with functionalized surfaces specific

for adsorption of the targeted substance of the multicomponent liquid.

All these problems will be discussed in the next sections that deal with bioreactors and downstream processes. Now, we focus on design and synthesis of magnetic biocompatible particles.

4.2. Magnetic beads – how to make them?

Magnetic beads can be manufactured using inorganic materials or several synthetic and natural polymers. High mechanical strength, thermal stability, solvent resistance and microbial attacks are commonly desired features. The easy manufacturing and the excellent shelf make the inorganic materials ideal supports. The main disadvantage of inorganic supports is their limited functional groups for selective binding: the most useful method is the adding of reactive organic functional groups in silane coupling.

Magnetic beads are more commonly manufactured from polymers because they have a variety of functional groups that can be tailored to suit specific applications (Griffiths and Bosley 1993, Safarikova and Safarik 1995, Demirel et al.

2004a, Elwakeel et al. 2009). The techniques described for the production of magnetic carriers can be used for variety of polymers. The polymer used as a binding or coating agent will depend on the final applications.

The carriers must fulfill several criteria related to their shape, size, rigidity, porosity, chemical inertness, density, magnetic properties, hydrophilicity, surface charge, surface concentration of reactive groups, cost, ease of manufacture, sterilizability, aggregation properties and mechanical strength (Pieters et al. 1991). Several comments on the supports prepared by different authors and commercial analogs have been published (Munro et al. 1977, Hailling and Dunhill 1980, Setchell 1985, Goetz et al. 1991, Pieters et al. 1991, Moffat et al. 1995, Safarik et al. 1995, Webb et al. 1996, Bozhinova et al. 2004, Ma and Liu 2007).

The methods for their preparations have been classified into four groups: direct use of a coupling agent, adsorption, encapsulation and formation of a thin film of polymer. Figure 28 illustrates the main concepts for magnetic bead preparation.

Either coating or entrapping magnetic particles in a polymer matrix can generally produce magnetic beads (Figure

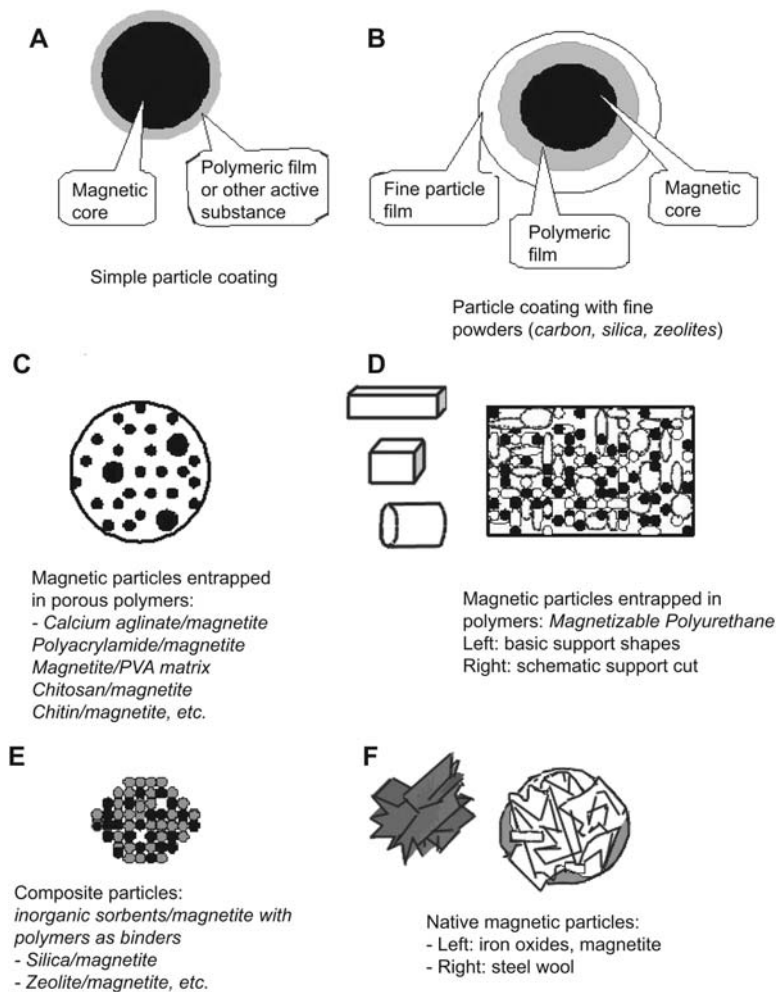


Figure 28 Basic principle to create magnetic supports. Adapted from Hristov and Ivanova (1999).

28C). One of the most popular and suitable magnetic materials is the magnetite, Fe_3O_4 . Typical polymers used are polyethyleneglycole (Bolto 1980), polymethylene-methacrylate (Rye and Bovin 1997), polyvinyl alcohol (Hu and Wu 1987, 1988) and polystyrene (Iman et al. 1992).

Magnetic beads based on gels are popular due to their smooth surfaces and large surface area for immobilization. The most used gels are polyacrylamide (Pollack et al. 1980, Burns and Graves 1985, Wu et al. 2010) and alginate (van Leemputten and Horisberger 1974a,b, Hailling and Dunhill 1979, Birnbaum and Larsson 1982, Lin J et al. 2009, Zhou ZD et al. 2010). However, the gel-based (the gels are biopolymers) beads are temperature-unstable and less resistant to pH and to solvents than polymer-based ones. Some authors have applied gel hardening using glutaraldehyde.

The magnetically susceptible supports can be separated in two main groups: porous and non-porous beads.

Porous magnetic beads These can be created using different conventional materials applied for biosupports or chemical adsorption. Some examples are listed below:

- Magnetite distributed in crosslinked polysaccharide (chitin, agar, agarose, κ -carrageenan, dextran) beads and magnetite/calcium alginate mixture (Molday and Mackenzie 1982, Burns and Graves 1987, Tilcock et al. 1988, Hamaya et al. 1993, Safarik and Safarikova 1993, Griffiths and Bosley 1993, Safarik et al. 2008, 2009, Zhou ZD et al. 2010).
- Chitosan/polymer encapsulating magnetic particles (Hritcu et al. 2009, Guo et al. 2010, Yang K et al. 2010, Zhou ZD et al. 2010, Zhou LM et al. 2010, Zhu et al. 2010).
- Aminoalkylsilylated magnetite (Sada et al. 1981b).
- Poly(methyl methacrylate) doped by magnetic particles (Pollert et al. 2006, Chen et al. 2008, 2009, Guo et al. 2009, Qu et al. 2009), poly(methyl) methacrylate magnetic latex (Hong et al. 2009).
- Magnetite included in polyacrylamide gel (Ivanov et al. 2009), thermo-sensitive polystyrene beads (Sada et al. 1980, 1981a,b, 1983, Kondo et al. 1994), composites based on vinyl template (Tudorachi and Chiriac 2008).
- Macroporous polystyrene beads (Griffiths and Bosley 1993, Yang CL et al. 2006, Yang K et al. 2010), magnetic polyurethane (Ivanov et al. 1997), duolite-polystyrene composite particles (Demirel et al. 2004a).
- Encapsulation of nanoparticles in swelling polymers by diffusion penetration (Chung et al. 2007, Chung and Lee 2008), miniemulsion polymerization of styrene (Zhang QY et al. 2007a,b, Joumaa et al. 2009), spraying suspension polymerization (Yang CL et al. 2005).
- Thermoresponsive polymers matrices doped by magnetic particles (Lin CL and Chiu 2005, Lin CL et al. 2006, Hoshino et al. 2007).
- Alumina particles impregnated by ferrofluids (Slavova et al. 2009).
- Low-cost magnetic nitrocellulose (Tanyolac and Ozdural 2000a) and polyvinylbutyral microbeads (Tanyolac and Ozdural 2000b).

Non-porous magnetic particles These seem to be more resistant to diffusional limitations, attrition and fouling than the porous supports (Ivanova et al. 1996). A variety of non-porous magnetic materials have been used, including iron, cobalt and their oxides (Munro et al. 1977), some examples include:

- Silica containing magnetite (Goetz et al. 1991, Safarik and Safarikova 1999a,b).
- Magnetic cores covered with a non-porous layer (Al-Hasan et al. 1991, Ivanova et al. 1996, Ozturk et al. 2007, Can et al. 2009, Gelbrich et al. 2010).
- Stainless steel spheres (Sieggell 1987), epoxy resin mixture with magnetite (Estevez et al. 1991).
- Complex fictionalization of surfaces of nanoparticles (Uygun et al. 2009, Li D et al. 2010).
- Composite sorbents of inorganic ion-exchangers (John et al. 1997, Sebesta 1999, Yang LR et al. 2009).

Ferrofluid treated cells and plant tissues draw a new trend in the preparation of magnetic biosupports. Ferrofluids containing well-suspended particles of nanoscale are well known in physics and technical applications (Odenbach 2003), but only recently successful chemical (Larachi 2007, Larachi and Desvigne 2007, Hajiani and Larachi 2009) and biochemical applications (Mosiniewicz-Szablewska et al. 2007, Safarik and Safarikova 2007, 2009, Safarikova et al. 2009, Ji et al. 2010) with great potential as biosupports and bioadsorbents (Safarik et al. 2005) have been suggested. All these applications will be commented on later in this review.

4.3. A brief overview of the main applications of magnetic beads

Affinity chromatography magnetic supports with covalently immobilized affinity ligands have proved to be effective in cell biology for affinity chromatography and human, animal and plant cell separation (Molday and Mackenzie 1982, Burns and Graves 1985, 1987, Tilcock et al. 1988, Terranova and Burns 1989, 1991, Williams et al. 1991, Kondo et al. 1994, Safarik et al. 1995).

The ability to separate and sort specific cell type from a cell population is an important tool in cancer research, transplantation and the study of the immune system (Burns and Graves 1985, Moffat et al. 1994, 1995). Highly selective separation is achieved using the reversible complex formation between two or more interacting biological species. Enzymes are coupled specifically with substrates, inhibitors, activators and coenzymes (Safarik and Safarikova 1993). Antibodies bind to antigens, hormones to receptors, lectins bind to specific surface antigens, red blood cells and carbohydrates (Kondo et al. 1994, Moffat et al. 1994) and magnetite-agarose beads for protein adsorption (Tong and Sun 2003).

Magnetic solid-phase extraction (John et al. 1997, Safarikova and Safarik 1999, Sebesta 1999), where a desired component is adsorbed onto the magnetic adsorbent recoverable from the suspension by appropriate magnetic separators, was developed as a very effective separation technique. The principle of magnetic solid-phase extraction is the batch

preconcentration of low amount of target analytes on magnetic adsorbents. After subsequent elution, very low concentrations in ppb ($\mu\text{g/l}$) range of some compounds can be detected (Safarik and Safarikova 2002b,c).

Magnetic (bio)sorption can be performed when fine magnetic seed particles of magnetite and silanized magnetite are attached to yeast cell wall envelopes, making them magnetic and amenable to separation. These envelopes were further crosslinked using epichlorohydrin and their biosorption characteristics (uptake of copper, cadmium and silver from aqueous solution) were determined by Patzak et al. (1997), Fogarty et al. (1999) and Ji et al. (2010). In a similar way, chitosan magnetic beads were developed for heavy metal removal (Rorrer et al. 1993, Elwakeel et al. 2009, Huang GL et al. 2009a,b, Huang HY et al. 2010, Zhou LM et al. 2010). The listing of applications will not be dealt with here, because most of them refer to heavy metal removal from aqueous solutions and impart to the next Part 9 of the series addressing separation processes.

Enzyme immobilization on magnetic beads such as microbial protease and amylases on magnetic calcium alginate (Burns and Graves 1985), cellulase on silanized magnetite (Garcia et al. 1989), *Aspergillus niger* pectinase on magnetic latex beads (Tyagi and Gupta 1995) and glucose oxidase, urease and bacterial α -amylases on paramagnetic polystyrene carriers (Garcia et al. 1989) are mainly studied in the field. Ferromagnetic beads with various immobilized enzymes were used in reactions accompanied by gas absorption: glucoamylase on κ -carrageenan/magnetite beads (Moffat et al. 1995, Webb et al. 1996, Wang et al. 2007) and gel containing ferrite particles (Sakai et al. 1994, 1999a,b), urease on polyacrylamide/magnetite (Sada et al. 1980, 1981a,b), glucose oxidase on polyacrylamide/magnetite (Varlan et al. 1996, Sohn et al. 2008), fish trypsin immobilization on magnetic Dacron (Amaral et al. 2006, Ivanov et al. 2009), invertase on magnetic Dacron (Cadena et al. 2010), pectinase on magnetic duolite-polystyrene composites (Demirel et al. 2004a) and many others.

The movement of the beads due to an external magnetic field (Sada et al. 1980, 1981a,b) can reduce the mass transfer resistance at the gas-liquid interface. In fluidized bed reactors (see the next section of this review) with immobilized enzyme magnetic particles, the dilution rates can be increased without appreciable effusion of the particles (Sada et al. 1980, 1981a,b). The magnetic field has been employed to control the movement of immobilized magnetic supports to avoid downstream processes (Sada et al. 1981a,b). More examples of enzyme immobilization on magnetizable carriers are summarized and reviewed by Safarik and Safarikova (1997).

Limited information is available about microbial cell immobilization on magnetic supports (Sada et al. 1981b, Ivanova et al. 1996). Hu and Wu (1987, 1988) studied the phenol biodegradation in model wastewaters with cells immobilized on polyacrylamide/magnetite beads. Cells of *Rhodopseudomonas sphaeroides* and *Alcaligenes eutrophus* immobilized on magnetite were applied for removal of environmental contaminants (Mac Rae 1985, 1986).

Saccharomyces cerevisiae cells were immobilized in a magnetite-containing alginate gel (Larsson and Mosbach 1979, Birnbaum and Larsson 1982, Weng et al. 1992, Sakai et al. 1994) or adsorbed on non-porous magnetic supports (Al-Hassan et al. 1991, Ivanova et al. 1996) and used for ethanol production. The paper of Bramble et al. (1990) is the unique study on plant cell (*Coffea arabica*) immobilization on magnetic beads.

Erythrocytes, magnetotactic bacteria, magnetoliposomes, magnetizable cells and microspheres are magnetic particles without applications in large-scale biotechnological processes but successfully working in microdevices (see later in this review). Erythrocytes can be separated by a high-gradient magnetic field. This needs a preliminary conversion of the diamagnetic erythrocytes containing oxyferrohemoglobin into metahemoglobin of paramagnetic red blood cells due to oxidation of the iron atom of the cell hemoglobin to the ferric state (Atef et al. 1995, Kim et al. 2005).

Magnetotactic bacteria due to the presence of ferrimagnetic material in their cell can be applied directly without magnetic labeling. Moreover, they have desired surface functional groups for selective binding, e.g., enzyme immobilization (Matsunaga and Kamyia 1987, Sun et al. 2008, Guo et al. 2010a).

Magnetoliposomes are magnetic derivatives of ordinary liposomes by incorporation of colloidal magnetic particles into the lipid vesicles (Safarik and Safarikova 2002a). They are usual carriers for drug targeting (Martina et al. 2008, Morais 2008, Chen YJ et al. 2010) or immobilization of membrane-bounded enzyme (De Cuyper and Joniau 1993).

Yeast cells have also been made magnetizable by a reversible adsorption of ultra-fine maghemite ($\gamma\text{-Fe}_2\text{O}_3$) particles on the cell walls (Dauer and Dunlop 1991, Safarikova et al. 2009, Fakhruddin et al. 2010, Garcia-Alonso et al. 2010).

The particles commented here cannot be termed “beads”, but they have a great potential for applications. However, at present they are applicable in small laboratory units and microdevices (Safarik et al. 1995, Safarikova and Safarik 1995, 1999, Safarik and Safarikova 1997, 1999a,b, 2004). Further comments in the present paper will consider the bioprocessings with magnetizable magnetic beads only.

4.4. Magnetic carrier arrangements in bioreactors

The biosupports arranged as particles of size 1 to 2–3 mm are conventionally used in packed beds or fluidized beds for bioprocessing. The magnetic field applied around the working volume does permit easy particle removal from the suspension in the downstream or an enhanced mass transfer control in the upstream process stages.

With regard to the particle concentration in the reactor, the known applications can be divided into two main groups (also see classification section):

- Low particle concentration reactors below 20%. These types of reactors are of a laboratory scale and the working volume does not exceed that of a flask. The earlier reports were done by Sada and colleagues (1980, 1981a,b, 1983).

The term “low-density concentration” reactors is also used.

- High particle concentration reactors with bed voidage (packed or fluidized regime) in the range of 40–70%. These reactors utilize the attractive features of MFA fluidized beds. They can also be termed MFA “fluidized bed reactors” or “high-density concentration” reactors.

These terms are equivalent to “low-density load” and “high-density load” introduced earlier in the classification section. Owing to the significant differences between both groups they will be discussed separately in the following sections.

5. Bioreactors with low-density loads of magnetic supports

The reactors with low-density load of magnetic biosupports are a step in the development of magnetically assisted bioreactors with magnetic carriers. The main research was done in the 1980s of the last century. Even though they are no more than shaking flasks surrounded by magnets, they will be reviewed because this step is necessary for correct understanding of the techniques employing magnetically controlled biocarriers.

The main concept is the use of a revolving (Sada et al. 1980) or switching (Sada et al. 1981a,b) magnetic field to assure a vigorous mixing, those eliminating the application of a mechanical stirrer and an appreciable reduction of the liquid-phase resistance. The electromagnets used were arranged to move the particles along longitudinal (Sada et al. 1981a,b, 1983) or transverse (Sada et al. 1980) symmetry axis of the reactor. Some of these experimental set-ups are illustrated schematically in Figure 29. The Sada group studies together and some separate investigations will be analyzed.

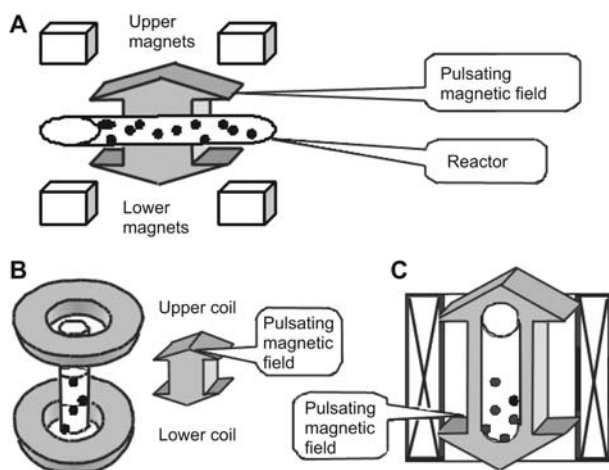


Figure 29 Basic concepts of the enzymatic reactors of Sada. Adapted from Hristov and Ivanova (1999).

5.1. The Sada group studies

Under the magnetic forces the particles were alternatively suspended and sedimented by revolution. The Sada group investigated urea conversion by urease immobilized on magnetite/polyacrylamide beads. Three versions of the reactor design have been investigated. The principle results will be reported briefly below.

Sada et al. (1980) A glass enzyme reactor (4.3 cm ID and 1.9 cm in height) was arranged with four electromagnets alternatively switched on at predetermined time intervals (Figure 29A). Magnetite entrapped together with urease in crosslinked polyacrylamide was used to prepare particles of 180 μm and 230 μm and a density of approximately 1200 kg/m^3 . The beads were suspended by a magnetic field at a revolving speed of 42 and 82 rpm and a magnetic field of 700 Gauss. The immobilized and soluble urease was also assayed in a stirred vessel with a blade stirrer at 610 rpm. In the reactor stirred by a magnetic field, the reaction rate has been the same as in a mechanically stirred vessel. However, the particle attrition due to collisions with the blade has been avoided.

Sada et al. (1981b) A transparent acrylic resin column (12 mm ID) has been placed in an electromagnet consisting of two coils (Figure 29B) generating a field with an induction of 0.09 T. The coils have been alternatively switched at predetermined time intervals. The beads (like that of Sada et al. 1980) with immobilized urease (90 μm in diameter) have been suspended and sedimented under the alternating magnetic field. The experiments performed repeated some of those reported by Sada et al. (1980). The average velocity (with regard to the stagnant liquid) has been reported to be approximately 5.0 cm/s. On that basis, by means of the Michaelis-Menten equation, the liquid-phase resistance was calculated as a function of time. No differences with the data obtained with conventional mechanical stirrers have been reported. The only advantages reported by the authors are the absence of collisions with the blade and that the magnetic field effectively reduces the liquid-phase resistance of substrate transfer.

Sada et al. (1981a) The experimental set-up is the same as that of Sada et al. (1981b) and beads (120 μm in diameter) the same as those of Sada et al. (1980, 1981b) were used. The column was placed inside a coil (Figure 29C) and a glass fritted plate was used as a supporting grid. The particles were fluidized by the liquid together with a simultaneous switching of the magnetic field (similar to Sada et al. 1980). Moreover, the magnetic field increased the effusion velocity of the particles from 3 cm/min in the absence of a field up to 24 cm/min at a magnetic flux of 900 Gauss. It was detected that the rates of conversion at low-liquid flow rates decreased with the increase in the magnetic forces. The authors attributed the fact to particle aggregation under strong magnetic forces. The results of this study demonstrate that the conversion rates were greater than those in fixed bed states (table 1 in Sada et al. 1981a). Similar behavior was

demonstrated with data of liquid-side volumetric mass transfer coefficient, K_{La} .

Sada et al. (1983) Glucose oxidase and catalase immobilized on crosslinked polyacrylamide/magnetite beds (similar to Sada et al. 1981a) were used. The experimental set-up is shown in Figure 29. There are no new results with regard to the mass transfer rate. The result is similar to those obtained in the absence of a magnetic field.

5.2. Other reactor designs

Sakai et al. (1994) used an alternating magnetic field of high frequency (at 5000 Hz) and intensity of approximately 200 Oe for the enhancement of ethanol formation by immobilized yeasts of *S. cerevisiae*. The experimental set-up is shown schematically in Figure 30. Iron powder and Ba-ferrite were used to produce magnetic alginate biosupports of 3.5 mm in diameter and density of 1200 kg/m³. The experiments revealed that the ethanol concentration increased by 12% with the immobilized yeast containing 50% iron powder and 200 Oe due to eddy currents. This effect corresponded to a 4°C increase in the temperature in the gel. A similar result was obtained with Ba-ferrite beads: 14% increase in the ethanol concentration at a frequency of approximately 2000 Hz and field intensity of approximately 400 Oe due to magnetic hysteresis effects. The same concept for reactor design and processes was developed by Sakai et al. (1999a,b) for enzyme reactions.

Comments on MFA low particle concentration bioreactors The investigations on bioreactors with low particle concentration and MFA bioprocessing do not indicate a systematic approach. The size and the concept of Sada's reactors do not allow application at a larger scale. The reason is that the magnetic system design reaches difficulties towards the scale-up. Generally, the studies of the Sada group indicate that the magnetic field can increase particle retention in the working volume at high liquid velocity. The magnetic field eliminates the mechanical stirrer, thus avoiding particle attrition and collisions with the blade. No effects of the magnetic field intensity on the reaction rate on the volumetric mass transfer coefficients (liquid-side) were detected.

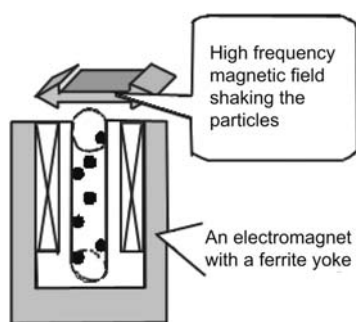


Figure 30 Basic concept of the reactor of Sakai et al. (1994) with a high frequency field causing hypothermia in the supports.

6. Bioreactors with high-density loads of magnetic supports

6.1. Basic concept and requirements

First, A brief introduction to the concept of using magnetizable biosupports in fluidized bed reactors is provided. In fact, the main concept is the same as that employed in magnetically assisted chemical reactors: control of the magnetic solids and control on the fluid-solid interphase heat and mass transfer. The repetition of some key elements is oriented mainly to those of the readers who are not familiar with the MAF and chemical reactors. Most of the publications on magnetic carriers stop at the moment of the preparation of solids and their characteristics. Then, as a rule, the application is not successful, because magnetic fluidization is unknown. This is simply explicable because most of the people working on materials and bioprocesses are not familiar with the bioreactor design and problems in the reactor development and improvement. Some of the following comments are available in the review of Hristov and Ivanova (1999), but owing to the publisher policy this article is not well attended by readers. Hence, after this brief rationale short explanations are provided: why should the biosupports be magnetic and operate in magnetically controlled fluidized beds?

The conventional bioreactors with biosupports suspended in the culture medium operate in two main modes: fixed bed and fluidized bed. It is well known that the bioreactors with immobilized cells and enzymes can benefit from the immobilization on carriers. First of all, the enzymes are water-soluble and second the immobilized cell activity is higher with regard to the free suspended cells. Thus, the immobilization allows easy separation of the enzymes and the cells from the liquid phase for multiple or a continuous operation as well as enabling the enzymes and the cells to be retained in working volume. Moreover, there are no enzyme or cell releases in the product stream. Usually, the high volumetric reaction rate with very small particles is the desired reactor design. However, the decrease in particle size leads to low minimum fluidization velocity and poor particle retention in the bioreactor. Thus, the optimal bioreactor design must be based on simultaneous satisfaction of two conditions: high reaction rate and high minimum fluidization velocity.

Most of the materials widely used for biocarriers have densities that are in the range of 1000–3000 kg/m³ which predetermines low liquid velocities and low mass transfer rates in a packed bed regime. Moreover, with near neutral buoyancy particles (densities of approximately 1000–1200 kg/m³) in a fluidized bed the particle elutriation cannot be avoided. The enhancement of the mass transfer efficiency together with the process performance in a packed bed must involve engineering solutions to hinder particle mixing at the desired liquid flow rates. The particle retention by a top-placed screen leads to undesirable high-pressure drops across the bed.

An alternative engineering solution is to make the particles “controllable” and “sticky”. The magnetic susceptible biocarriers allow easy particle aggregations under the action of

an external magnetic field and easy break-up of the aggregates when the magnetic field is switched off. Thus, a bed of magnetically “sticky” particles must remain free of mixing motions (in a packed bed state) at velocities higher than if the stickiness does not exist. The second possibility is to reach a fluidized bed of particles aggregates that continuously form and break up without particle elutriation. Thus, the solution is: application of a magnetic field holding up and controlling the particles in the reactor.

The main question that arises is about the reason requiring the magnetic field application. It is evident that the increases in the particle-fluid slip velocity decreases the liquid-side mass transfer resistance. In other words, the intensive mixing diminishes the diffusion resistance in the liquid phase. This leads to enhancement of the mass transfer resistance and increased efficiency of the bioprocessing. The results of Sada (Sada et al. 1980, 1981a,b, 1983) commented on above clearly demonstrate this effect. Moreover, these studies demonstrated that the magnetic field holds up the magnetizable beads in the working volume, thus preventing their escape due to higher fluid velocity. From an engineering point of view the question could be set up as follows: How to combine the features of both the fluidized and packed beds and those of the magnetizable solids?

As mentioned, the mass transfer rate increases as the particle size decreases. However, the disadvantage of using small particles is that they can then be difficult to recover from the process liquid by means of conventional sedimentation or centrifugal operations due to the high solution viscosity and additional colloid particles commonly existing in the food industry. Thus, magnetizable solids do permit easy separation from the process solution using well-known magnetic separation techniques (Williams et al. 1991).

The conventional packed bed and fluidized bed arrangement needs a column with supporting grid and the necessary auxiliaries supplying the fluidizing liquid or gas (Yates 1983, Kunii and Levenspiel 1991). The new element of the bioreactor with the magnetic field acting on the solids is the magnetic system. From a general point of view, this device must generate a magnetic field that acts over the whole working volume of the reactor. Therefore, these reactors are analogous to the encircled free-cell reactors with the principal difference – the cells are immobilized. The main concept comes from the MAF, i.e., to hold the particles at high dilution rates of the liquid phase. Moreover, the possibility to control the motion of the solids with immobilized microorganisms offer new opportunities in the downstream processing (see the next section). Furthermore, new phenomena have been discovered that enhance the efficiency of these new bioreactors both for upstream and downstream processing. The basic concept and main features will be commented on step-by-step in the following sections.

6.1.1. Magnetic field effects on fluidized magnetic biosupports When magnetic biocarriers are in the reactor and undergo fluidization, there are no differences in their behavior with regard to the system already reviewed in the previous part of this series. The only fact that can be pre-

dicted is the lower fluid velocity, due to the reduction in weight of the solids (composites of low-density biomaterials suitable for immobilization and high-density but inert magnetic particles) and predominately operation in two-phase liquid solid systems, and to some extent in 3-phase (G-L-S) beds. Hence, the basic characteristics of magnetically assisted fluidized beds are not addressed here but it is recommended, prior to starting this section, to read Part 5 (liquid-solid bed), Part 6 (gas-liquid-solid fluidization) and Part 7 (mass transfer: chemical reactors). However, some specific points concerning bioreactor designs and relevant magnetic systems will be dealt with below.

The magnetic field applied on the particle bed causes significant interparticle attractive forces if the beads are magnetically susceptible (Part 1 and Part 5). The interparticle attraction will hinder the free movement of the particle inside the bed (Abbasov et al. 2010). This becomes important beyond the velocity corresponding to the minimum fluidization in the absence of a magnetic field. The magnetic forces hinder the intensive particle movement and delay the fluidization. The bed height increases incrementally by the slow increase in the liquid velocity, but without particle mixing motions. This delayed fluidization is the principal attractive phenomenon provoking the development of the bioreactors with a magnetic field. It is termed “magnetic stabilization” (see Part 1 and Part 5) and has several attractive features:

- high fluid-particle slip velocities are possible;
- fixed bed regime without particle collisions and bead attrition is possible at high fluid velocities;
- easy separation of the liquid and solid phases by means of the magnetic field action;
- easy control of bed internal structure (particle arrangement) thus enhancing the mass transfer processes;
- with conducting liquids there exist additional hydromagnetic effects influencing the chemical reactions similar to those exhibited in conventionally packed beds under magnetic field exposure (Munteanu and Larachi 2010).

6.1.2. Magnetic field generation The source of the magnetic field acting on the bead in the bioreactor can be created in different ways. Usually the investigators used solenoids (Figure 31) or short coils well known from the textbooks of physics. Each of these devices generates a magnetic field with lines of force parallel to the symmetry axis. This type of field is termed “axial field” (Part 1). For example, some of the Sada group investigations were performed in such fields (Figure 31). This field has been used by Raja Rao et al. (1997) for a circulating free-cell bioreactor (Figure 25). The other opportunity is to create a field transverse to the symmetry axis of the working volume similar to the study of Sakai et al. (1994). Until 1990 transverse fields have been generated by electromagnets with iron yokes or permanent magnets (see Part 1 for details). They have been used mainly for gas-fluidized beds except the unique investigations of Burns and Graves (1988). These magnetic systems have limited applications owing to difficulties emerging during the scale-up. Sometimes there are investigations with such

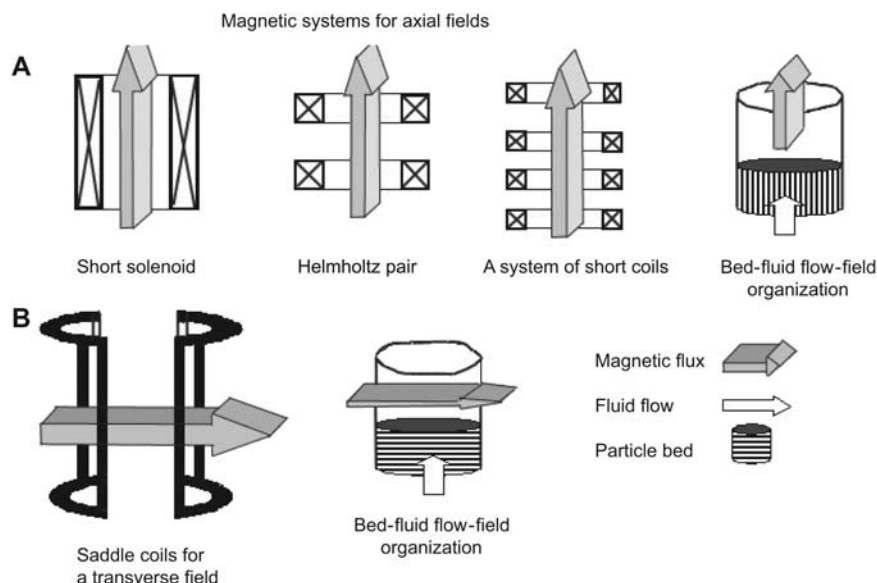


Figure 31 Magnetic fields used and relevant magnetic systems to create them for magnetically assisted bioreactors. Adapted from Hristov and Ivanova (1999). (A) Axial fields and magnetic system used to generate them. Inset: Bed-fluid flow-field organization. (B) A transverse field magnetic system created by two saddle coils. Inset: Bed-fluid flow-field organization.

devices, but the working volume does not exceed several cubic centimeters. After 1990 a new magnetic system for a transverse magnetic field has been conceived for the purposes of the MFA fluidization (Figure 31B) and successfully applied to bioreactors (Ivanova et al. 1996). The system is based on two saddle coils generating a homogeneous magnetic field transverse the longitudinal symmetry axis.

6.1.3. Fluidization modes of magnetic field assisted fluidized beds The magnetic field effect on fluidization depends both on the intensity and on the orientation of the field lines. The fluid flow and the magnetic field can be applied independently, thus two modes of operation are possible (Part 1):

1. Magnetization FIRST mode. This mode involves the application of the field on a fixed bed and fluidization after that. Siegel (1987) has introduced the term. The description of bed behavior done above corresponds to that mode.
2. Magnetization LAST mode. The second mode involves the application of the field on the preliminarily fluidized bed.

The interesting phenomenon for the bioprocessing is the particle arrangement in the magnetically stabilized bed (MSB) which allows operation in a fixed bed regime beyond the point of minimum fluidization in the absence of a field. This regime can be achieved in the magnetization FIRST mode only. The structure of the MSB is strongly affected by the field line orientation. The magnetic particle aggregates in slender aggregates oriented along the field lines (Part 1). The aggregation cannot be eliminated, because it is a magnetic analog of Newton's law of gravity. Sada et al. (1980, 1981a,b, 1983) reported the same phenomenon in reactors

with low particle concentration. Beyond the point of minimum fluidization, when the stabilized bed is broken, the system becomes fluidized. The next section briefly addresses only liquid-solid beds as dominant in biotechnology.

The main phenomena and regimes in liquid-solid fluidized beds attractive for bioprocessings include:

- MSB regime due to the absence of particle mixing in the velocity range corresponding to the fluidized state in the absence of field. The absence of particle collision and attrition processes are attractive for biological beds of soft and fragile biosupports with immobilized cells and immobilized enzymes.
- Fluidized bed regime due to intensive particles mixing, mainly for applications with immobilized cells (see applications).

In both regimes the efficiency of the mass transfer operation depends on the field lines orientation (see below). The two points above are interesting for upstream processing. The MSB offers unique properties that allow the creation of new separation devices (see section on Downstream processes).

6.2. Two-phase (liquid-solid) bioreactors (anaerobic)

The MFA fluidization has been applied for basic biotechnological applications: enzyme-immobilized processing and cell-immobilized processing. These will be discussed separately.

6.2.1. Enzyme immobilized processes

6.2.1.1. Carriers for enzyme immobilization The enzyme carriers were described briefly in section 4 (see Main applications of magnetic beads). Taking into account the specific

feature required for enzyme processing adsorption, covalent attachment or entrapment in a porous bead might immobilize enzymes.

The adsorption-based immobilization has advantages of easy regeneration and multiple use of the magnetic carrier. The enzyme adsorption is possible for enzymes that possess as a low isoelectric point and optimal pH. For example, α -amylase has a pH optimum of approximately 5–6. The choice of a suitable magnetic carrier must be precise in order to prevent desorption under operating conditions (Munro et al. 1977).

Magnetic beads allowing a covalent bonding of enzymes are usually based on coated magnetic cores. The coating materials are polymers derivatized with specific reagents (Pieters et al. 1991). They are under intensive investigations and allow a formation of a group of low-cost enzyme immobilization techniques with carrier-sized in the colloid range.

The co-precipitated polymer-magnetite beads are in the range of the submicron sizes. The common methods are based on precipitation of polymer containing solution of ferric chlorides at pH 10 and 70°C (Molday and Molday 1984). Several polymers can be used, including:

- chitosan (Burns and Graves 1985, Rorrer et al. 1993),
- dextran (Molday and Mackenzie 1982) or
- PEG derivatives (Inada et al. 1988).

It is important to control the size distribution of the final particles, because the population of the magnetite particles varies with the production conditions (agitation rate, pH and temperature). Fluidized bed applications are desirable mono-sized distributions that can be achieved by a post-preparation classification.

6.2.1.2. Processes – examples Two types of magnetic enzyme processes are known: batch and continuous. The MFA fluidization allows process creation in both modes. The particles and the solution can be separated by means of the external magnetic field retaining the former in the reaction volume. Thus, expensive separations by high gradient magnetic systems (Williams et al. 1991) can be avoided.

A) Investigations in University of Manchester Institute of Technology (UMIST)

Experiments

Moffat (1993) performed a maltodextrin hydrolysis as a model enzyme reaction because the process is mass transfer limited (Allen et al. 1979, Freire and Santanna 1990, Kovalenko et al. 2006). Support particles of κ -carrageenan/magnetite produced by an emulsion polymerization technique were used for glucoamylase immobilization with a density of approximately 1060 kg/m³. Supports (κ -carrageenan/magnetite) of 0.37–0.92 mm in diameter were used.

Several laboratory reactors employing axial magnetic fields were designed (Figure 32). 1) Magnetic system of four coils for columns of 113 mm ID (bed height of 62 mm (Webb et al. 1996) and 50 mm ID and 100 mm ID for con-

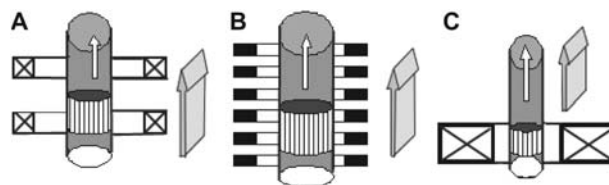


Figure 32 Magnetically assisted enzyme reactors developed in UMIST. Adapted from Moffat (1993) and Hristov and Ivanova (1997). (A) Helmholtz pair with a small volume reactor. (B) Short coils systems for a large (tall) reactor. (C) Short solenoid used by Webb et al. (1996).

tinuous processing. 2) Two-coil system for a column of 14 mm ID for batch experiments. All the experiments were carried out in the MSB regime (i.e., magnetization FIRST mode). In addition, the experiments were performed consequently in batch and in continuous modes and with low and high substrate concentrations (3% w/v and 35% w/v maltodextrin).

The batch experiments of Moffat demonstrate that there is no magnetic field effect on the maltodextrin with low substrate concentration (3% w/v) hydrolysis at field induction of 10 mT (Figure 33A,B). Moreover, it was confirmed that the process is under the limitation of the external mass transfer because the increase in the substrate velocity increased the conversion. The field of 10 mT retained all the particles in the bed, because the working velocity was approximately 0.875 mm/s. The minimum velocity enough to entrain the particles was 0.67 mm/s, thus without the field all the particles were entrained into process liquor. At 0.875 mm/s substrate velocity, the glucose yield was approximately 88%.

The hydrolysis of 30–35% w/v maltodextrin was carried out because the industrial processes use this concentration of the substrate. The observations showed that the process was impossible without the application of a magnetic field. The bead density (1060 kg/m³) was lower than that of the maltodextrin (1070 kg/m³). The higher substrate density led to increased field induction required for bed stabilization (15 mT). Because of that increasing, the magnetite content has increased the bead density to 1100 kg/m³ (particle diameter=150–500 μ m).

Moffat's batch experiments (Moffat 1993) show that the increase in the substrate velocity from 0.175 mm/s to 0.35 mm/s leads to an increase in glucose conversion from 91% to 96% (Figure 33A,B) in a batch operating mode. The data indicate that there could be a benefit associated with the magnetic stabilization due to the lack of particle mixing, which, in fact, increases the lifetime of the enzyme.

The continuous maltodextrin hydrolysis was carried out with 35% w/v maltodextrin conversion to glucose. The magnetic field affects non-significantly the conversion [figure 8.14 in Moffat (1993): from 125 kg/m³ at zero field conditions to 137 kg/m³ at 10 mT and to 160 kg/m³ at 20 mT approximately]. Despite this, Moffat pointed out that the glucose conversion was high (38%) due to the stabilizing effect of the substrate.

Increased bed loading experiments (Figure 34) demonstrated that the increase in the particle amount from 0.2 kg

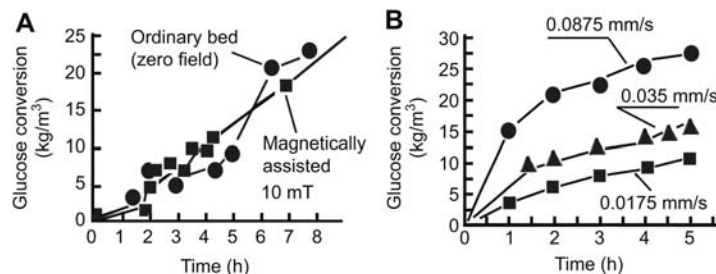


Figure 33 Moffat's results on 3% (w/v) maltodextrin conversion glucose in MFAB. Reactor diameter: 14 mm ID. (A) Magnetic field effect on the conversion yield-batch mode (adapted from figure 8.9 of Moffat 1993). (B) Substrate velocity effects in a recirculating MFAB (adapted from figure 8.10 of Moffat 1993). Courtesy of Prof. R. Williams (Leeds).

to 0.6 kg increased the conversion to 60%. Furthermore, in a bed containing 3 kg a 79% conversion of maltodextrin to glucose was attained. This is an improvement of the conversion values published for fluidized beds: 76% (Allen et al. 1979) and 15% (Vallat et al. 1985) where very small quantities of biosupports were used. Moffat (1993) pointed out that the results of MFAB are significantly lower than 96% conversion required for industrial applications. The experiments performed with lower particle size (the bead diameter was changed from 260 μm to 188 μm) demonstrated a conversion augmentation to 86%. This reduction in the conversion might be attributed to decreased fluid-solid contact surface as a result of the particle aggregation as well as reduced fluid-solid slip velocity and then increase in the diffusion mass transfer resistance. The latter is the most probable reason, because increases in the fluid velocity when the particle size is reduced require stronger fields. Moffat (1993) applied the same field intensities to beds of coarser particles.

The scale-up studies did not detect effects of reactor diameters on the conversion rate (Figure 34) continuous MFAB, but comments on the coil diameter were done.

Mass transfer model

Moffat (1993) pointed out that there are no mass transfer equations developed for the case of MFAB, due to its com-

plicated hydrodynamic behavior. Then, Moffat used the classic Michaelis-Menten mechanism:

$$-\frac{dC}{dt} = \frac{V_{\max}C_s}{K_m + C_s} \quad (5)$$

and correlated the mass transfer rate (Moffat et al. 1995, Webb et al. 1996) as

$$\frac{K_{GP}}{U} \varepsilon Sc^{\frac{2}{3}} = f(\varepsilon) \left(\frac{U d_p}{\nu} \right)^{-0.5} \quad (6)$$

where

$$f(\varepsilon) = (1 - \varepsilon)^{0.5} \quad (7)$$

is the function related to moderate bed porosity and

$$f(\varepsilon) = 0.66 \varepsilon^{0.5} \quad (8)$$

for high bed porosities.

The net force on a particle in the reactor has been expressed as balance of both the magnetic and the hydrodynamic forces (Moffat et al. 1995):

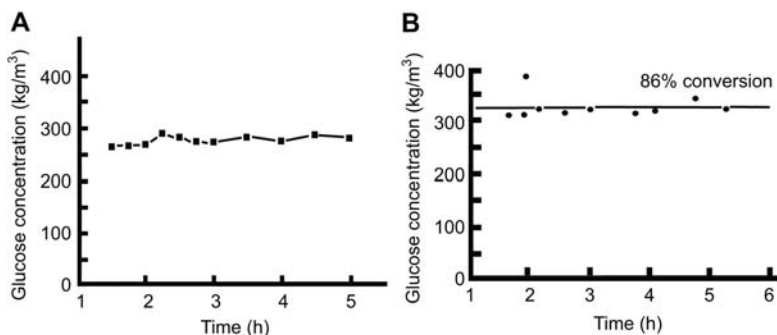


Figure 34 Moffat results obtained in 0.1 m ID column (continuous maltodextrin conversion). Courtesy of Prof. R. Williams (Leeds). (A) Batch experiments: bead loading effect on the conversion; 3 kg beads, $d_p = 200-500 \mu\text{m}$, $U = 0.1 \text{ mm/s}$; figure 8.18 of Moffat (1993). (B) Continuous process (figure 8.19 of Moffat 1993), $d_p = 150-300 \mu\text{m}$, $U = 0.2 \text{ mm/s}$.

$$F = 12\pi d\mu \frac{U}{(1-\varepsilon)} - \left(\frac{\pi}{6}\right)gd^3(\rho_p - \rho_L) - K^* \cdot H(H-H_p)A_p. \quad (9)$$

On the basis of Eqs. (5) to (9), the reactor behavior was described. Figure 35A,B show the model predictions. However, this model has limitations, notably because it is based on empirical terms that have not been tested for immobilized enzyme applications.

B) Other studies

Bahar and Celebi (2000) immobilized *glucoamylase* (EC 3.2.1.3. from *Aspergillus niger*) on magnetic polystyrene particles including active groups and tested a fluidized bed reactor operating in an axial magnetic field (short solenoid) (Figure 36A). Maltose (10% w/v) in 6.3 1022 M NaAc buffer solution with pH 4.5 at 50°C was used as substrate. The operating mode was magnetization LAST because the only goal was to keep the particles (Bahar and Celebi 2000) of very low density (average diameter of 125 μm) in the column. The effect of the internal bed structure was not at issue in this study. Finally, the mass transfer studies were performed in different regimes emerging in the bed: from completely fluidized bed at zero field to a “frozen bed” (see Part 1 for the definition) at high field intensities. Figure 36B shows the immobilized enzyme activity as a function of the magnetic field intensity.

These results clearly demonstrate the effect of the bed internal structures on the mass transfer efficiency. Even

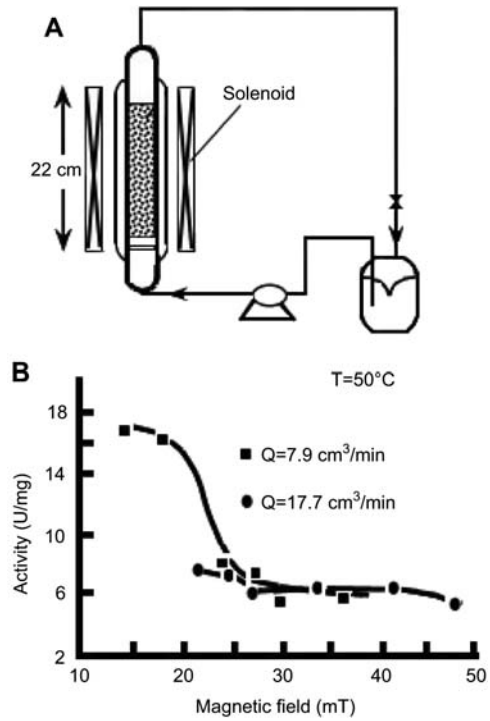


Figure 36 Experiments on Bahar and Celebi (2000) with immobilized glucoamylase on magnetic polystyrene particles. (A) Experimental set-up. (B) Enzyme activity as a function of the magnetic field applied. In fact, this a performance of the bed structure on the mass transfer represented by the enzyme activity.

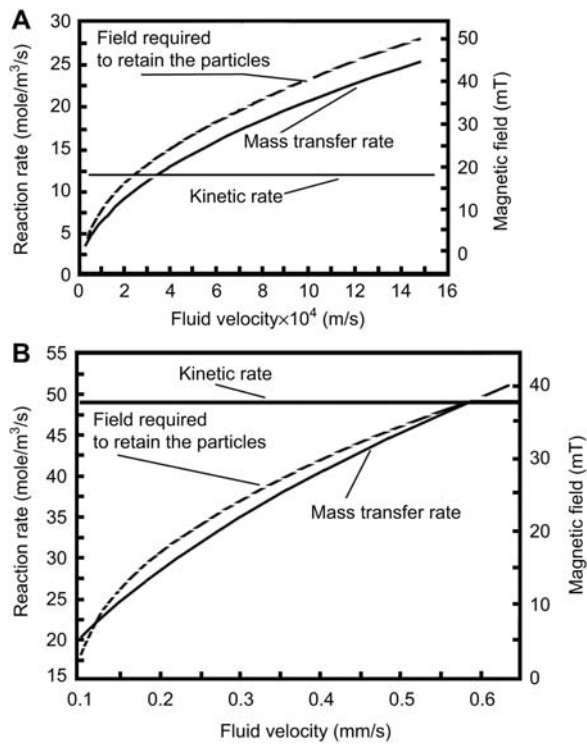


Figure 35 Model prediction of the bioreactor reaction rate at different flow rate. (A) According to Moffat (1993). (B) According to Webb et al. (1996).

though high slip velocity can be attained in the frozen bed regime, its poor anisotropic structures with channels and strongly aggregated particles result in significant fluid bypass and reduced fluid-solid mass transfer area. The effect of the reduced mass transfer area is clearly demonstrated by the high enzyme activity at low-field intensity, where despite the decreased fluid-solid slip velocity larger contact area is available due to decreasing effects of particle aggregations. The latter simply means that the aggregates external surface is lower, whereas the fluid and enzyme in the internal part of the aggregates practically do work those results in low performance of the reactor. The main problem in this study comes from the inappropriate choice of the magnetization mode. The magnetization FIRST mode, i.e., the MSB regime is more suitable for fixed bed regime operations when owing to the low densities of the particles the fluidized mode is practically impossible. The MSB structure at moderate expansions has more developed fluid-solid contact area, undeveloped channels and moderate particle aggregations.

Demirel and colleagues (Demirel and Mutlu 2004, Demirel et al. 2004b) performed a systematic study with Pectinex Ultra SP-L immobilized on magnetic duolite-polystyrene particles (Demirel et al. 2004a) in both fixed bed mode (Demirel et al. 2004a,b) and undergoing fluidization in a magnetic field (Demirel and Mutlu 2004):

The fixed bed (FB) experiments (Demirel et al. 2004b) with particles (212 μm and 425 μm) The Michaelis-Men-

ten model and the Lineweaver-Burk plots of experimental data for free and immobilized enzymes revealed that the Michaelis-Menten constant K_m is higher for the immobilized enzyme (1.877 g pectin/ml) than that observed with the free enzyme (0.515 g pectin/ml). The corresponding maximal activities are: 5.669×10^{-3} g pectin/s $\cdot\mu$ l enzyme (immobilized enzyme) and 1.047×10^{-3} g pectin/s $\cdot\mu$ l enzyme (free enzyme).

The tests in a magnetically assisted reactor (Demirel and Mutlu 2004) with 10 mm ID and 120 mm in height revealed that the ability to increase the flow rate resulted in reduced enzyme activity (approximately 40% reduction for 5 times increase in the liquid flow rate). This range is somewhat wide for MAF and span fixed bed, MSB and the onset of fluidization (Part 1 and Part 5). As the flow rate increases, the structure becomes more anisotropic, the fluid bypass is dominant, together with reduced fluid-solid contact area that resulted in reduced apparent enzyme activity. In fact, these experiments are almost the same as those of Bahar and Celebi (2000) because in both studies inappropriate bed structures were created. Under these conditions the apparent parameters of the Michaelis-Menten model were determined through a simple model developed on a mass balance across a slice of the bed. For immobilized enzyme: $K_m = 4.416$ g/l and $V_{max} = 5.5 \times 10^{-5}$ g/s $\cdot$ g particles, whereas those of the free enzyme were 0.439 g/l and 44.8 g/l, respectively. The ability to reuse the immobilized enzyme because by the magnetic field the particles are retained in the reactor was tested. The reduction in the enzyme activity was approximately 75% at the end of the third run. No more results exist in this article with regard to optimal reactor conditions and immobilized enzyme performance.

6.2.1.3. Comments on magnetically assisted processes with immobilized enzyme The systematic search and analysis of the published information yield the above review comments with disappointing outcomes, including:

- 1) For approximately 20 years (1993–2010) only two systematic studies on magnetically assisted bioreactors with immobilized enzymes were performed: UMIST (Pieters et al. 1991, Williams et al. 1991, Moffat 1993, Moffat et al. 1994, 1995, Webb et al. 1996) and that in the Hacettepe University, Turkey (Bahar and Celebi 1999, 2000, Demirel and Mutlu 2004, Demirel et al. 2004a,b).
- 2) This is a disappointing result in time when there is a mass of publications on magnetic particles for biosupports (see section 4). Unfortunately, the upper level in applications of the developed magnetic biosupports for enzyme immobilization in most of the published articles are:
 - 2a) Fixed bed regime with its limitation in the fluid flow rate imposed by the particle low-density and the minimum fluidization velocity (Inada et al. 1988, Akgol et al. 2001, Bayramoglu and Arica 2008a,b; and many others from above).
 - 2b) Fluidized bed regime with low slip velocities and limitation from the above on the operating fluid flow rate (Rorrer et al. 1993, and many others).
- 3) There are no attempts to apply the techniques of the MAF despite its advantages. The reasons for this situation include some principle drawbacks of the already published results, including:
 - 3a) Axial fields were only applied with consequent particle orientation along the field lines and the limited zone with homogeneous field distribution. The inappropriate magnetization (magnetization LAST mode) creating an unfavorable internal bed structure which, in fact, is the worst existing one in magnetic fluidization and therefore unusable for mass and heat transfer operations. This led to low performance of magnetically assisted enzymatic processes with regard to the conventional fluidized bed regime (Bahar and Celebi 2000).
 - 3b) Missing attempts towards adequate scale-up and choice of better magnetic systems. The studies of Webb and colleagues (Pieters et al. 1991, Williams et al. 1991, Moffat 1993, Moffat et al. 1994, 1995, Webb et al. 1996) indicate that owing to additional forces created by the external magnetic field the control of particle movement and liquid flow rate through the reactor is available. The processes were performed in the MSB (magnetization FIRST mode). The conclusions of Moffat (1993) showed that the scale-up faced serious problems due to the unpleasant scale-up of the magnetic system used. This common disadvantage of the axial magnetic systems will be discussed later.
 - 3c) Missing adequate approaches in the bioreactor design and many impractical ones for engineering design laboratory studies in small vessels. Analysis of reactor performance, typical of the chemical engineering approach, is generally missing in the published articles. All these facts, mainly due to the author's experience, qualifications and viewpoints, resulted in actuality that magnetically assisted bioreactors are considered as inappropriate and strange among the biotechnological society. However, this is not true and if the reader can read this review and the preceding reviews (Part 1 and Part 7, for example), it will be motivating to develop the research in the right way and to obtain adequate and valuable results.

6.2.2. Cells – immobilized processes

6.2.2.1. Liquid-solid fluidized bed bioreactors with immobilized cells Here we will consider only liquid-solid reactors. The three-phase counterparts will be reviewed in the next section. The separation allows elucidating the bed hydrodynamic effects and processing organization of the bioreactor performance, irrespective of the process itself: aerobic or anaerobic. Details are summarized in Tables 2–4.

In general, the studies on two-phase processes in magnetically assisted bioreactors (Bramble et al. 1990, Ivanova and Hristov 1995, Ivanova et al. 1996, Ivanov et al. 1997) were performed with the magnetization FIRST mode. The main

Table 2 Details about experimental studies on cell immobilized two-phase magnetically assisted bioreactors.

Process	Magnetic system	Column	Beads	Cells	Liquid	Mode	Ref.
Caffeine and theobromine production	Four coils	$D_c = 50$ mm $L_c = 520$ mm	Ca-alginate/ Fe_3O_4 $d_p = 2.1$ mm; $\rho_s = 1080$ kg/m ³	<i>Coffea arabica</i>	Oxygenated substrate	Magnetization FIRST	Bramble et al. (1990)
Ethanol production	Saddle coils (figure 38)	$D_c = 40$ mm $L_c = 1000$ mm	Activated carbon covered magnetic cores $d_p = 1.2$ mm; $\rho_s = 3300$ kg/m ³	<i>Saccharomyces cerevisiae</i>	Glucose substrate	Magnetization FIRST	Ivanova et al. (1996)
Ethanol production	Helmholtz pair (figure 40A) $H_{max} = 20$ kA/m	50×50 mm Square cross-section column $L_c = 1000$ mm	Polyurethane/magnetite foam Cubes $3 \times 3 \times 3$ mm $\rho_s = 1100$ kg/m ³	<i>Saccharomyces cerevisiae</i>	Glucose substrate	Magnetization FIRST	Ivanov et al. (1997)

Table 3 Variation of magnetic polyurethane properties affecting the fluidization property of the bed.

% (Mass) magnetite sand	Density kg/m ³ (wet)	Density kg/m ³ (dry)	Porosity
37.8	890	64	0.93
39.2	920	71	0.85
71.4 ^a	1100	175	0.84
72.6	1108	146	0.85
76	1146	201	0.83
21.4	660	45	0.93
28	978	65	0.92
42.9	1010	92	0.89

^aUsed in experiments.

reason for this operating mode is the fact that the regime of MSB avoids the bead attrition and cell release in the culture medium but at the same time ensures expanded structure, increased fluid-solids mass transfer area and reduced external diffusion mass transfer resistances due to increased slip velocity. Moreover, the fixed structure of MSB allows a control of the gas produced in situ during the process (CO_2 or other gases), thus preventing undesirable particle mixing and collisions. Details are summarized in Table 2.

The first publication in the 1990s belongs to Bramble et al. (1990) on plant cell immobilization in MSB. The subsequent studies (Ivanova and Hristov 1995, Ivanova et al. 1996, Ivanov et al. 1997) focused on bead compositions and bead arrangement effects on ethanol fermentation.

6.2.2.2. Processes – examples

A) Plant cell immobilized magnetically assisted reactor

Bramble et al. (1990) reported the unique investigation on plant cells (*Coffea arabica*) on Ca-alginate/magnetite beads in an axial magnetic field. Bramble conceived the two solid flow operating modes (see later): a conventional batch mode and a continuous downflow moving particle bed. The former mode has only been investigated. Despite the fact that a hollow fiber cartridge has oxygenated the liquid medium (deionized water) before its inlet in MSB, the process is considered here as liquid fluidized. The reason for this assumption is the absence of large gas bubbles similar to the three-phase beds. Moreover, the gas-to-liquid mass transfer occurred before the MSB inlet (an oxygen K_{La} value of 62.4 h was reported.). This study shows that there is a lot of engineering problems facing the creation of successful MFA bioreactors, including the following.

Table 4 Details about experimental studies on cell immobilized G-L-S MFA bioreactors.

Process	Magnetic system	Column	Beads	Cells	Liquid	Gas	Mode	Ref.
Phenol degradation	Helmholtz pair $B_{max} = 50$ mT	$D_c = 30$ mm $L_c = 500$ mm	Polyacrylamide/ Fe_3O_4 4.5% (wt) wet cells and 30% Fe_3O_4 (wt) $d_p = 3.6$ mm; $\rho_s = 1080$ kg/m ³	No data	Phenol contaminated water	Air	Magnetization LAST	Hu and Wu (1988)
Ethanol production	Short solenoid $H_{max} = 105$ Oe	$D_c = 150$ mm $L_c = 1000$ mm	Ca-alginate/ Fe_3O_4 $d_p = 2.1$ mm; $\rho_s = 1080$ kg/m ³	<i>Saccharomyces cerevisiae</i>	Molasses	Air	Magnetization LAST	Weng et al. (1992)
Acetic acid production	Helmholtz pair $H_{max} = 20$ kA/m	50×50 mm Square cross-section column $L_c = 1000$ mm	Zeolite covered magnetic cores $d_p = 1.2$ mm; $\rho_s = 3100$ kg/m ³	<i>Acetobacter aceti</i>	Ethanol and water	Air	Magnetization FIRST	Ivanov et al. (1997)

First of all, the effect of magnetite content as well as the concentration of the entrapped cells on the intraparticle diffusion coefficients of the beads was studied. No such investigations have been reported in the previous studies and little information on the problems exists even in the modern era. Bramble's results are shown in Figure 37. The results demonstrated that any diffusion problems were due to the size of the beads and the biochemical reaction rate, not to the bead composition.

Second, the immobilized cell cultivation in MSB shows that the caffeine production (5 mg/l at day 20) is lower than that in a shaker flask (10 mg/l at day 19). The experiments (four successful experiments only) indicated that magnetite inhibits caffeine production (confirmed by separate experiments). A similar result was obtained for the theobromine production: 7.0 mg/l in a shaker flask in contrast to 5.0 mg/l in the MSB. These results indicate that something was not well organized in the MSB reactor. The better results in the shaker reveal reduced external mass transfer resistance. Two potential problems lead to low production:

- too expanded bed with formed channels and aggregates with entrapped stagnant liquid;
- insufficient mixing of the oxygen and the liquid prior to the entrance at the reactor bottom. The non-homogeneities in the bed structure could lead to formation of zones (pockets) rich of gas and poor in liquid and vice versa. In both cases of pocket formation, the mass transfer at the bed surface is reduced owing to insufficient nutrients supplied to the cells.

Third, no differences in the cell growth were observed, but the caffeine production was altered when the process shifted from the flask to the reactor. This fact was mentioned above and two potential reasons were given. Moreover, Bramble et al. (1990) explained the above results with the over-ventilation of the medium with air, which displaced the volatile components (ethylene, a plant hormone that is naturally produced by cells or CO_2). These results show that ethylene does not affect cell growth but greatly enhances the production of theobromine and caffeine. Therefore, the better experiments with the designed MSB reactor envisaged low

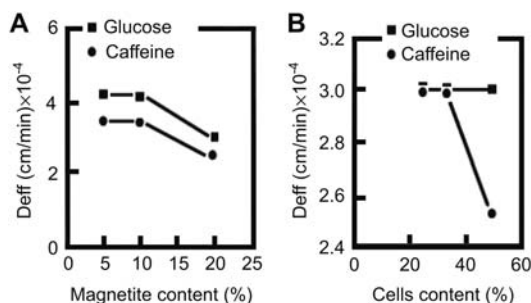


Figure 37 Effect of biosupport composition on the diffusion coefficient (data extracted from table 1 of Bramble et al. 1990). Adapted from Hristov and Ivanova (1999). (A) Effect of magnetite content at 1.5% alginate. (B) Effect of cells content at 10% magnetite. Note: diffusion coefficients in water: glucose – 4.8×10^{-4} cm/min; caffeine – 3.408×10^{-4} cm/min.

airflow rate or with ethylene addition to the aeration gas. At low gas, both over-ventilation and the pocked formations can be avoided. However, such experiments were not reported by Bramble et al. (1990).

The investigations from a hydrodynamic point of view are obscure and the published data do not allow elucidating the effects of bed structure on the process. The authors did not observe particle arrangement along the field lines and channeling similar to all other studies on MSB. This seems peculiar at present when too much is known about the MAF. But, in general, the style in the performance of the experiments and the level of knowledge of what exactly is the tool (magnetizable bed) used in the mass transfer process and how to operate with it, as well, are almost the same as those demonstrated by the studies on the enzyme immobilized processes. Bramble's experiments indicate that the application of MFA bioreactors needs compromise engineering solutions either on bead composition design or the process-operating regime, and better knowledge about what is the tool (the magnetic field) used for process performance.

B) Ethanol fermentation

Ivanova et al. (1996) and Ivanova and Hristov (1995) carried out ethanol fermentation with *S. cerevisiae* yeasts on non-porous beads (Al-Hassan et al. 1991) (Figure 38). Both investigations were performed in a transverse magnetic field in contrast to all other studies discussed here. The reason for the transverse field application is well illustrated in Figure 39. The axial particle arrangement in slender aggregates oriented along the field lines sets up a serious engineering problem as was commented in the case of enzyme immobilized processes. The channeling induced by the aggregates decreases the efficiency of the fluid-particle contact and the resulting mass transfer yield. A way to avoid the problem is aggregate orientation transversely to the fluid flow. Moreover, the magnetizable beads contain low amounts of magnetic substance (usually magnetite). This requires higher magnetic field intensities than in the case of beds consisting of pure magnetic materials as noted below. The fermentation medium was composed of 100–180 g glucose and the appropriate

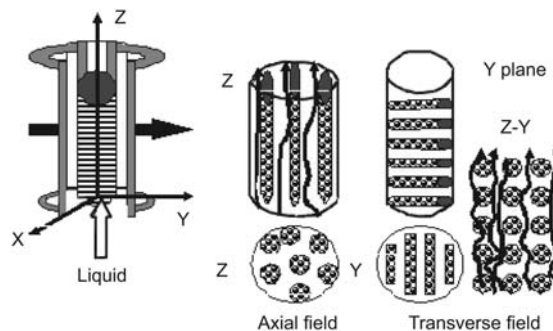


Figure 38 Experimental set-up of Ivanova et al. (1996) and basic particle arrangement. The particle arrangement transverse to the fluid flow leads to better fluid-particle contact (see cross-sections under two field orientations).

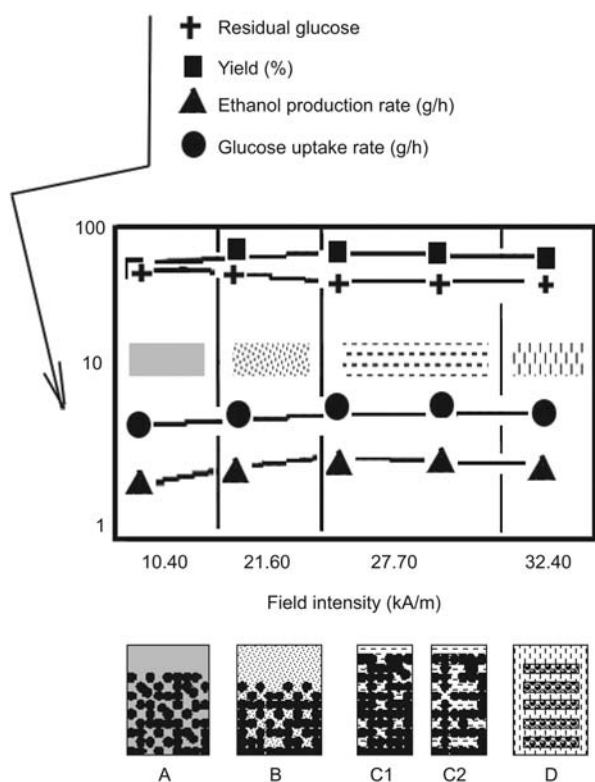


Figure 39 Field effects on the fermentation process. Adapted from Ivanova et al. (1996) and Hristov and Ivanova (1999). A – ordinary packed bed; B – MSB (expanded bed); C1 – MSB (highly expanded bed); C2 – highly expanded bed with cracks; D – MSB of separate particle aggregates (“strings”).

quantities of water and mineral salts. The experiments were carried out in the MSB regime. The main results are outlined next:

- The turn-on of the magnetic field (after 48 h cultivation without a field) allows the establishment of the desired liquid flow rate (60 ml/h) greater than the minimum fluidization velocity in the absence of a field. This increases the ethanol production of 1.5 times and the glucose uptake rate of approximately 115% (Figure 38). The ethanol concentration reached 59.0 g/l at 96% yield of the theoretical. The bioreactor productivity was 21.8 g/l/h and the residual glucose concentration decreased to 55–60 g/l (75–85 g/l) in a conventional packed bed, respectively.
- The investigations were performed with field intensities within the range of 10.4 to 27.7 kA/m. At higher field the released biomass increased from 1.0 to 1.5×10^7 cell/ml, the ethanol concentration was lower and the process efficiency dropped.

The experimental results were attributed to the bed structures available under the simultaneous action of the fluid flow and field. Figure 39 illustrates these effects, namely:

- Zone A. The field is not applied. The particle arrangement and the fermentation efficiency are similar to these in conventional packed beds.

- Zone B. The field intensity is low. The magnetic attractive forces are not enough to create oriented particle structure. The bed expansion is not significant, but the increase in the bed porosity increases the glucose conversion.
- Zone C. The interparticle attraction is enough to create a significantly expanded bed. The formation of particle aggregates and horizontal cracks are not possible. This bed structure assures the maximum fluid-particle contact area and the maximum fermentation efficiency. The fact is attributed to the internal bed structure only. From Zone A to Zone C the particle arrangement along the field lines leads to increased bed porosities and decreased diffusion mass transfer resistance.
- Zone D. The field intensity is high and the bed structure is highly anisotropic. The aggregates are similar to porous bars streamlined transversely by the fluid flow. This decreases the effective fluid particle contact area of the outer surfaces of the aggregates. At these surfaces the convection dominates, thus decreasing the diffusion mass transfer resistance. However, in the bulk of the aggregates the molecular diffusion dominates the convective transport. This explains the fact that both the glucose consumption and the ethanol production decrease.

Ivanov et al. (1997) developed magnetizable polyurethane foam as successful combinations of the well-known properties of polyurethane foam (Webb 1987) as biocarrier and the magnetizable biosupports. The magnetizable polyurethane foam is illustrated schematically in Figure 28. The main goal was to decrease the minimum fluidization velocity by decreasing the bead density and to create large pores enhancing the cell immobilization. This condition allows the dilution rate of the reactor to be decreased without change in the bed hydrodynamic conditions. Commercially available polyurethane foam (Sirolex Ltd, UK) was mixed with magnetite sand (0.1–2 mm, density 5100 kg/m³) to manufacture blocks in glass moulds (Petri dishes). Then cubes (3×3×3 mm) were prepared by cutting the blocks mechanically. Particular attention was focused on achieving bead density either lower or greater than that of the water (Table 3). The experimental set-up is shown schematically in Figure 40.

S. cerevisiae grown aerobically on yeast extract-peptone-glucose (YEPG) medium was immobilized by entrapment. The results on ethanol yield in time are shown in Figure 40. Three runs are presented: Run 1 (zero field), Run 2 ($H=10$ kA/m) and Run 3 ($H=20$ kA/m). The MSB regime was created within liquid superficial velocity of $U_L=1$ –4 mm/s that is twice lower than the velocity range in the study of Ivanova et al. (1996) with heavier carriers. The development of bed structure and bed voidage, respectively, promotes the ethanol production rate from 5.2 g/l/h (zero field) to 7.1 g/l/h at $H=10$ kA/m. The reduction of the ethanol rate production to 4.8 g/l/h was observed at $H=20$ kA/m. The latter result is due to channeling in the bed and resembles the effects of the bed structure (Zone D) in the experiments of Ivanova et al. (1996) noted above.

Liu CZ et al. (2009) carried out a study on ethanol fermentation by immobilized *S. cerevisiae* in magnetic Ca-alginate particles (3 mm in diameter) produced by mixing and

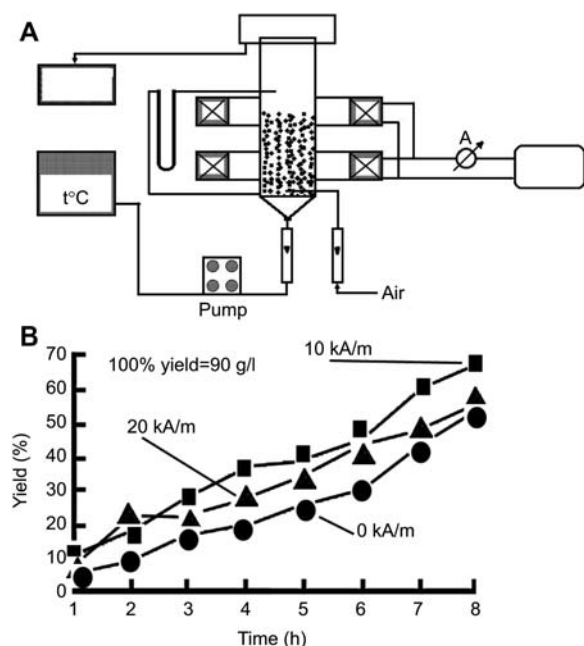


Figure 40 Continuous ethanol fermentation. From Ivanov et al. (1997). (A) Experimental set-up. (B) Fermentation runs at different field intensities. Note: the field intensity controls the bed structure at fixed fluid flow rate. For explanation of these results see the comments about the work of Ivanova et al. (1996) and Figure 39.

droplet (jet break-up by vibrating nozzle) stabilization in a CaCl_2 solution to complete the gelation. A reactor of 2.76 l operating under an axial magnetic field (a Helmholtz pair of two 10 cm thick coils with intensity of up to 500 Oe $\approx$ 40 kA/m) was used. During the fermentation process, the broth was drawn out from the upper part of the reactor and recycled back to the reactor from the bottom with a liquid circulation rate of 20 times faster than the fresh medium feed rate. The main results are outlined next.

The Liu's experiments with the particle preparation revealed that for application to magnetically assisted beds a compromise between the bead mechanical stability and the development of their intraparticle structure should be attained. The best results were obtained with particles gelled in 2% CaCl_2 concentration, with flexible internal structure and enough strength to maintain the conditions imposed by the magnetically assisted bed. The main reason for this optimization was provoked by the fact of unequal growth of the cells immobilized at the bed surface and those inside the pores. Insufficient nutrient delivery in tight pores of hard and inflexible alginate beads results in better growth of the surface immobilized cells than those in depth of the supports.

Continuous ethanol fermentation runs with a different amount of particles in the volume [26%, 32%, 41%, 51% (v/v)] and a fluidizing synthetic medium [90 g/l glucose at different field intensities (95–110 Oe) and a dilution rate of 25 h] were performed. In general, the ethanol productivity increases (Figure 41) with increase in the amount of the particles loaded in the reactor, but at 52% (v/v) there was a problem with the efficient removal of the CO_2 produced *in situ*.

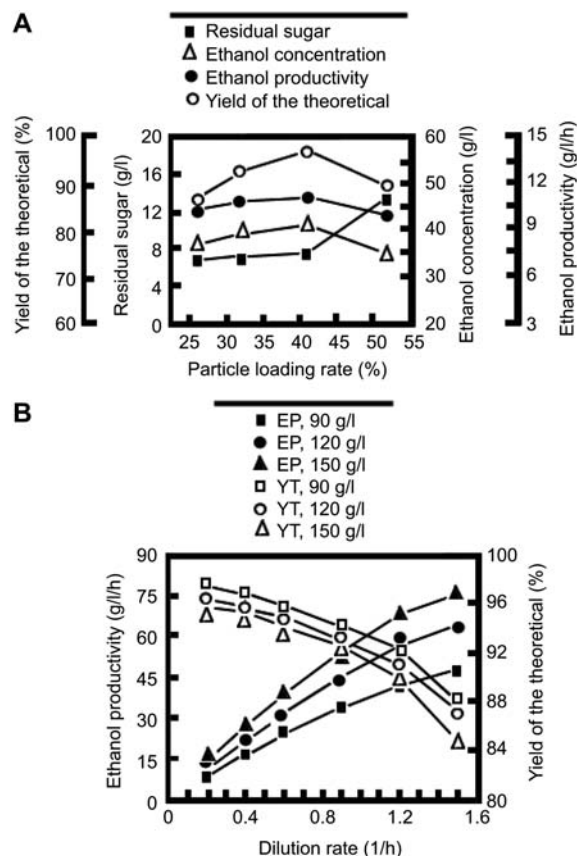


Figure 41 Ethanol fermentation experiments of Liu CZ et al. (2009). (A) Effect of particle loading rate on continuous ethanol fermentation in a magnetically stabilized fluidized bed reactor. (B) Dilution rate vs. ethanol productivity (EP) and yield of the theoretical (YT).

Synthetic media with different glucose concentrations (90, 120 and 150 g/l) were circulated at various dilution rates through a bed of optimal 41% (v/v) loading of the supports stabilized by fields in the range of 80–120 Oe. These experiments resemble (as conditions concerning the medium, field intensity and reactor volume) those performed by Ivanova et al. (1996).

The ethanol productivity in the magnetically assisted reactor with the immobilized *S. cerevisiae* cells increased with the increase in both the sugar concentration and the dilution rate. However, the increased dilution rate reduces the ethanol concentration, which is an expected result. At glucose concentration of 150 g/l the ethanol concentration and yield attained approximately 66 g/l, i.e., approximately 95% of the theoretical one. However, these results were obtained at dilution rates limited from above by the value of 0.4 h.

Similar experiments with continuous ethanol fermentation of molasses were performed under various conditions imposed by the particle load, the dilution rate and the field intensity. The runs provided results comparative to those obtained with glucose solutions. It was mentioned that efficiency of the magnetically assisted reactor was higher than

that observed in a conventional fluidized bed (zero field conditions).

In fact, the results confirm the data published by Ivanova et al. (1996). However, the use of the dilution rate as a process variable does not give accurate information about the conditions created in the bed. The bed heights attained under different dilution rates are missing in the work of Liu CZ et al. (2009). From the present author's experience and some approximate calculations based on the published data, it might be suggested that the upper dilution rate of 0.4 h corresponds to either a fixed bed regime (undeveloped bed structure) or MSB state, but at slow expansion. It is impossible to analyze how the bed structure affects the process performance as was done by Ivanova et al. (1996).

In general, the work of Liu CZ et al. (2009) confirms the fact that attaining slip velocities higher than those permitted in traditional fixed beds have the following benefits:

- the ethanol production is enhanced;
- the CO₂ liberation is better;
- the sugar consumption is augmented;
- the entire process performance is improved.

The axial field, however, limits the fluid flow rate due to the common disadvantages of the MSB created under such conditions: narrow velocity range without channeling and strong aggregate formation (see Part 1 and Part 5).

C) Bacteria immobilized on magnetic beads

E. coli and *B. subtilis* were immobilized by Estevez and colleagues (Estevez et al. 1990, 1995) to heavy carriers based on magnetite, sepiolite, epoxy resin and silicon oxide (see compound particles in Figure 28). The studies described the hydrodynamics of the supports in the presence of a magnetic field generated by a short coil and the bead compositions (Estevez et al. 1995). However, no data about the bioprocesses performed are available.

D) Biodiesel by magnetic cell biocatalyst based on *B. subtilis*

Ying and Chen (2007) encapsulated *B. subtilis* in hydrophobic magnetizable carriers containing magnetite particles. Then, the lipase produced by *B. subtilis* was conjugated with carboxyl at the magnetic polymicrosphere surface and used to produce enzymatic biodiesel from waste cooking oils. The particles were characterized as superparamagnetic. This allows easy separation by electromagnetic separator after 48 h of cultivation. When methanolysis is carried out using the magnetic carriers, the yield of methyl ester in the mixture was approximately 90% after 72 h reaction in a solvent free system. The methanolysis was carried out at 40°C, 220 rpm and pH 6.5 in a 100-ml shake flask incubating on a rotary shaker, not in a fluidized bed reactor.

This information was especially introduced even though MAF was not employed. One more step is needed to implement the "lipase production carriers" in a magnetically

assisted reactor. It could be expected that published results in this direction will appear soon.

E) Mammalian cell cultivation in MSB

At the end of this section, we have to mention the work of Mathew (1994) which never appeared as a journal publication. This is a very good thesis with results comparable to all well-completed studies discussed above. In fact, this is the only study where the problem of mechanical stresses in fluidized bed on the cell growth was discussed. As a reasonable solution a reactor operation in the MSB mode (axial field) was chosen. Gelatin/magnetite microgranules were used as biocarriers (100 µm and 425 µm). The particles were optically transparent that allowed the study of bed hydrodynamics and particle frequency (regarding potential stresses on the cells). The cultivation of mammalian cells in MSB assured minimal shear stresses. The cell death was especially studied in the exponential growth phase at a field of 70 Gauss. It was established that the cell death in MSB was approximately $4 \times 10^{-3}/h$, whereas in the ordinary fluidized bed it was approximately $5 \times 10^{-3}/h$. At the same time, the specific cell growth was approximately 0.0279 h and 0.024 h, for MSB and fluidized bed, respectively.

The mammalian cell proliferation was especially studied by Mathew (1994) but no differences were found in magnetized and non-magnetic cultured medium. Formation of cell aggregates was reported, but it contributed to the carrier surface properties, not to the magnetic field effects.

6.2.3. Common problems of liquid-solid fluidized bed bioreactors

The studies discussed show that the main effect of the magnetic field is the higher particle-fluid slip velocity available in the stabilized bed regime. The bed internal structure is strongly related to the slip velocity, higher bed porosity, higher slip velocity and higher mass transfer efficiency. Taking into consideration that the magnetic field imposes anisotropy in the particle arrangement the channeling should be avoided. The two possibilities investigated, axial and transversal field orientations, provide a basis for the reactor design. The transverse field allows greater liquid velocities, greater volume occupied by the bed, channeling elimination as well as low power consumption (see the discussion later). The second problem is the bead density. As mentioned in the comments on Moffat investigations, the small difference between the carrier and liquid densities causes problems. One of the solutions is to manufacture heavy particles, thus increasing the bed minimum fluidization velocity. An alternative is to produce lighter particles with low minimum fluidization velocity. In the former case, the reactor length increases parallel to the required dilution rate, whereas in the second situation the bed height might remain as in the conventional packed bed situation. There is a third possibility not yet investigated.

Even though the above comments were issued to the reader 10 years ago (Hristov and Ivanova 1999) little was done in the direction drawn. Practically there is no development in the implementation of magnetically assisted bioreactor

although there is a strong development of magnetic biocarrier. The only new investigation, but exploring an old concept, is that of Liu CZ et al. (2009) on ethanol production. There are too many problems in the implementation of magnetically assisted bioreactors but most of them are not due to missing technical solutions for design of carriers and reactors. Their deficiency is in the field of ideas of how to implement magnetic biocarriers, not only to produce them.

6.3. Three-phase (G-L-S) bubble column based bioreactors

The hydrodynamics of the three-phase magnetically assisted reactor was reviewed in Part 6. Here, we will comment on the studies on bioprocesses based on such devices. The order follows the chronology of the investigations starting with the first study of Hu and Wu (1987, 1988).

6.3.1. Overview with comments The first reports of Hu and Wu (1987, 1988) were on a three-phase magnetically controlled bioreactor using polyacrylamide magnetizable beads (the concept of Sada) for cleaning artificial wastewater from phenol. The main results of this first work on three-phase magnetically assisted bioreactors can be summarized as follows:

- Within the concentration range of phenol used there are no inhibitory effects on cells (cell type is not defined) growth.
- The magnetic field does not affect the rate of phenol degradation.
- The amount of phenol degraded increases with the magnetic field increase: from 0.406 g/l/h without a field to 0.680 g/l/h at field induction of 50 mT.
- The higher field intensities allow higher gas flow rates thus leading to larger amounts of phenol degraded. Thus, the volume loading of the fluidized bed contaminants can be increased with regard to the non-magnetic beds.
- The effect of the magnetic field on the mean gas hold-up (ε_g) is that $\ln(\varepsilon_g)$ depends linearly on the field intensity. The operating mode employed was magnetization LAST.

Weng et al. (1992) reported the second application of the MFA gas-liquid-solid bed as bioreactors. Continuous ethanol fermentation has been carried out with *S. cerevisiae* immobilized on alginate/magnetite beads. The column has been equipped with a magnetic distributor (see detailed comments in Part 6). The short coil of the distributor holds the beads in the reactor volume thus preventing their elutriation. Ethanol fermentation of molasses lasted 28 days with maximum effluent ethanol concentration of 9.0% v/v, bioreactor productivity of 14.2 g/l/h and molasses conversion of 90%. This study demonstrates that the particle aggregates penetrate into the bubbles and break them, and both bubble diameter and bubble rise velocity decrease with magnetic field strength. Under these conditions the volumetric mass transfer coefficient K_{La} has been expressed as:

$$K_{La} = K_L \left(\lambda + \frac{1}{A + b \cdot e^{-CmH}} \right) \quad (10)$$

or on the basis of the experimental data

$$K_{La} = 10^{-3} \left(8.61 + \frac{1}{0.1334 + 7.89 \cdot e^{-0.048H}} \right) \text{Re}_l^{0.0055} \text{Re}_g^{1.21} \quad (11)$$

$\text{Re}_L = \frac{d_p U_L \rho_L}{\mu_L}$ and $\text{Re}_G = \frac{d_p U_G \rho_G}{\mu_G}$ are the Reynolds numbers defined for the liquid and the gas phase, respectively. The field intensity is expressed in Oersteds (1 Oe $\approx$ 79.9 A/m).

Based on the above results, Weng et al. (1992) found that the bioactivity of the cells (10 cells per 1 ml gel) immobilized on alginate beads had the same bioactivity as those immobilized on alginate/magnetite beads. The macro-kinetics of the fermentation was described by a modified Michaelis–Menten equation

$$\gamma = \frac{137.98S}{77.98 + S} \left(1 - \frac{P}{124.23} \right) \quad (12)$$

A pilot-scale three-stage bioreactor (0.66 m ID) was designed on the basis of the above study (a short note by Weng et al. 1992) with an ethanol effluent concentration of 8–9% v/v and bioreactor productivity of 10–12 kg/m³/h.

The studies of Hu and Wu (1987, 1988) and Weng et al. (1992) were followed by the complete investigations of Kwauk et al. (1992) on the bed hydrodynamics. The principal results directly related to bioreactor performances include:

- The local gas hold-up depends on the field intensity and the radial coordinate.

$$(\text{Hu and Wu 1988}) \quad e_g = e_{g0} - 0.0122 \cdot U_g^{1.32} \cdot \Phi^{1.6} \quad (13)$$

$$\text{Weng et al. (1992)} \quad \varepsilon_g = \bar{\varepsilon}_g (1.878 - 0.0036H)(1 - \Phi^2) \quad (14)$$

- The liquid mixing coefficient has been described by a modified mixing model:

$$\frac{dC(t)}{dt} = \frac{[C - C(t)]}{Lh} U_L \quad (15)$$

with the initial conditions

$$t=0, C(t)=0 \quad (16)$$

The mixing coefficient in the solution

$$\frac{C(t)}{C_0} = 1 - \exp\left(-\frac{t}{\tau\eta}\right) \quad (17)$$

$$h = (0.1038 + 1.52 \cdot 10^{-5} H^{1.737}) U_G^{0.2518} \quad (18)$$

where the mixing coefficient h is affected by the field intensity in the range of 0–125 Oe.

Ivanov et al. (1997) employed the particles created by Al-Hassan et al. (1991) for immobilization of *Acetobacter aceti* strain. The acetic acid fermentation as a typical high oxygen consumption process was used for the reactor performance. The retaining action of the magnetic field allows high gas hold-up to be achieved. Figure 42 shows the acetic acid yield variations with the gas velocity. Three example curves are shown: Run 1 (zero field), Run 2 ($H=10$ kA/m) and Run 3 ($H=20$ kA/m). The bed operated in the MSB regime (magnetization FIRST mode) in contrast to the experiments of Hu and Wu (1987, 1988) and Weng et al. (1992). Part 6 of this review series provides details about the regimes in three-phase magnetically assisted reactors.

Ames and Worden (1997) developed a magnetically assisted bioreactor for daidzein and genistein from immobilized soybean culture for over 2 months. The bioreactor (50 mm ID and 60 cm in height) used an axial field created by a Helmholtz pair (no data are available). The soybean cultures were coimmobilized with ferritic stainless steel in calcium alginate spheres. Immobilized soybean concentrations reached over 10 g of dry tissue/l of biocatalyst. Daidzein and genistein concentrations ranged from 10 to 200 g/g of dry soybean tissue. The biocatalysts were investigated microscopically and it was decided that oxygen diffusion was the rate limiting factor. In addition, to some extent contaminations of the culture by Ampicillin, Timentin and Benlate with limited phytotoxicity in the continuous bioreactor operation were detected. This study provides too many details that might be valid to any other reactor operating without the magnetic field. The effect of the operating conditions created by the external magnetic field is completely missing. It might be suggested that the main concept was to hold the particles in the reactor only by application of an external magnetic field rather than to investigate the reactor performance. In fact, this study has the same disappointing effect as those on the enzymatic processes discussed earlier in this section. The reader cannot understand what purpose of the magnetic field is in the described process.

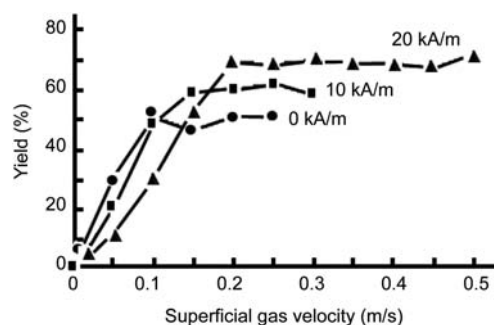


Figure 42 Continuous acetic acid production (Ivanov et al. 1997). Conditions: initial bed height = 100 mm, $U_L = 0.05$ mm/s; 100% yield = 40 g/l. Series: 1 – $H = 0$ kA/m, 2 – $H = 10$ kA/m, 3 – $H = 20$ kA/m.

Al-Qodah (2000) reported antibiotics production in a magnetically assisted (transverse field) bubble column. The hydrodynamics of this device was discussed in Part 6. Al-Qodah presented the problem from two positions: 1) modeling and 2) experiments. The biosupports for immobilization of sensitive filamentous microorganisms *Penicillium chrysogenum* were the same as those reported by Al-Hassan et al. (1991), i.e., magnetite cores covered by carbon and natural zeolites (Note: Z. Al-Hassan and Z. Al-Qodah are one and the same person appearing under different names in the publications).

The model simulation sufficiently predicted the concentration of lactose in the solution. In addition, the experimental results reveal increases in the penicillin production with increases in the field intensity from 0 to 35 mT. Al-Qodah attributed the positive effect to the increase in K_{La} in a magnetically controlled bed which was mainly as a result of the possibility to keep the bubbles small in size. This yields increased consumption of lactose as the field intensity was increased. All these effects are of hydrodynamic origin and the control on the bubble size and effective culture aeration.

6.3.2. Briefs on G-L-S magnetically assisted bioreactors

The studies noted above (see the data in Table 4) clearly demonstrate that the hydrodynamic effects on the mass transfer in magnetically assisted bioreactors are of primary importance. The field only allows the hydrodynamics to be enhanced that logically results in a better mass transfer performance of the magnetically controlled bioreactors. The mean gas hold-up, bubble diameters and the volumetric mass transfer coefficient K_{La} can be somewhat effectively controlled by the field controlling the particle motions and the aggregate formation of the particle aggregate size.

Unlike the magnetically assisted chemical reactors (see Part 7) it is not possible to record significant development in the area. In the first decade of the new millennium there are no new articles on three-phase magnetically assisted bioreactors. To some extent, the reasons for this are common with those discussed with regard to the three-phase processes.

7. Micro and nanoscale magnetically assisted biodevices

7.1. Foreword

At the beginning of MAF the technique envisaged only macroscopic devices and all laboratory level studies were oriented towards improvement of existing technologies. However, in the modern rapid development of technologies oriented toward micro and nanoscales the need of magnetically assisted processes emerged again. The main and foremost reason is that by external magnetic field it is possible to control particle movement, fluid flow and heat/mass transfer at micro-level, where any mechanical devices known

from the well-established techniques are impractical. The area of microsystems for performance of heat and mass transfer operations is rather complex and encompasses phenomena, techniques, force fields, laws and scales that are negligible in everyday technologies (Kim et al. 2005, Lund-Olesen 2005, Dobson et al. 2006, Li Y et al. 2007a,b, Liu J et al. 2007, Ceylan et al. 2008, Korecka et al. 2008, Spero et al. 2008, Silva et al. 2009, Kosoy 2010, Lin H et al. 2010, Schiavone et al. 2010). However, decreasing the scale, the new world exhibits its own laws and rules. Only those applications where magnetically assisted devices are used for upstream processing and the design and modes of operation resemble those in the macroreactors will be encompassed.

First of all, the magnetically assisted microreactors operate with fine magnetic particles and miniature magnetic systems. In general, the concentration of particles is low, thus they resemble the systems developed by Sada et al. (1980, 1981a,b). The fluidization is magnetically driven as a rule, because the fluid flow rates are too low to suspend the particles and create fluidized beds. Some cases are discussed next without any attempt in this section to do a general overview of the topic which is rather large, difficult as a task and beyond the scope of this review.

7.2. Micro MFAF reactor with magnetic biocarriers

One very typical magnetically assisted microdevice was recently developed by Yang K et al. (2010) in a 1.5-ml microfuge tube with coimmobilized enzymes (glucoamylase and α -amylase) onto chitosan particles containing gamma ferric oxide particles for starch liquefaction and saccharification under the magnetic field. A key motivation behind this study was to develop immobilized enzymes for applications in analytical/diagnostic microdevices in small reaction vessels with or without mechanical agitation. The magnetic chitosan beads were fabricated using the emulsion/crosslinking technique of Denkbass et al. (2002) in the range of 150–300 μm .

Different external electromagnetic fields (DC, single-phase AC, and three-phase AC) were used by combination of the coils of a set consisting of two or six short solenoids (57.2 mm ID, 20 mm in height) equidistantly (16 mm apart) arranged as Helmholtz pairs used to generate various types of axial magnetic fields. The system was generated by a single-phase (60 Hz) AC or a DC magnetic field. To generate a three-phase AC magnetic field (60 Hz), six coils were wired together via a delta configuration to form a three-phase winding. To reciprocally change the traveling direction of the magnetic field produced by the three-phase coils, a programmable timer was used. In all experiments the strength of the external magnetic field was adjusted to 330 Oe ($10\text{e} \approx 79.9 \text{ A/m}$).

The reactor was placed between two adjacent magnetic coils, charged with magnetic chitosan beads (50 mg) with immobilized enzyme and incubated with 1 ml starch solution. The highest reaction rates were achieved using a three-phase AC field, followed in a close second by single-phase AC, then DC, and no external magnetic field (Figure 43A).

The changing in the polarity every 1 s in the three-phase AC did not lead to further improvement in the reaction rate (Figure 43A). The initial reaction rate under the AC field was calculated to be approximately 1.7 and 4.1 times faster than that in a DC field and at the condition without a magnetic field, respectively. Under the single-phase AC field (60 Hz), the magnetic beads tumbled forcefully owing to the constantly changing field polarity. This required that the beads have proper coercivity and remnant magnetization. Superparamagnetic particles (having zero remnant magnetization) will not be able to tumble under a single-phase AC field. These results strongly indicate that intensive mixing reducing the external diffusion resistance augment the mass transfer rate (Figure 43B). The static field blocks the particles, forms clumps and reduces the fluid-solid contact area. The change in the polarity in the experiments reveals misunderstanding of the field action. In fact, it works like a magnetic field of an electric motor. Generally, this is a magnetically driven fluidization (see the definition in Part 1) where the fluid is stagnant and the driving forces are generated by the magnetic field. Such reactors are limited in the amount of particles charged because an increase in the number of particles blocks their motions and they form an immovable clump. From this point of view, the work of Yang K et al. (2010) is a new version of the concepts of Sada et al. (1980, 1981a,b, 1983). Yang K et al. (2010) noted that the AC magnetic field can enhance intraparticle mass transfer as a result of magnetic powder vibration, such enhancement should sustain even under high stirring rates. However, the particles are fixed in the chitosan matrix and such strong internal vibrations could affect the mechanical stability. Most likely, the observed improvement of the interparticle mass transfer was due to eddy currents in the conductive liquid pockets and in the ferric particles. The effect is new and should be studied in detail. The reactor of Yang K et al. (2010) is only among the microreactors utilizing a magnetic field that copies the concepts of the large-scale MAF.

7.3. Various magnetically assisted microreactors

7.3.1. Microreactors with magnetic inductive heating

Microreactors assisted by a magnetic field to control the inside temperature by inductive heating of functionalized magnetic nanoparticles was developed by Ceylan et al. (2008). The concept of magnetically induced hyperthermia based on specific properties of magnetic nanoparticles upon exposure time-varying magnetic field was developed. A principal problem associated with naked metallic nanoparticles is their high chemical reactivity, in particular oxidation by air. Because of this, magnetic silica-coated nanoparticles were used inside a microfluidic FB reactor for performing chemical syntheses including catalytic transformations. Ceylan et al. (2008) demonstrate that in addition to conventional and microwave heating, magnetic induction in a moderate-frequency electromagnetic field (25 kHz) is also a method to introduce thermal energy to the reactor volume.

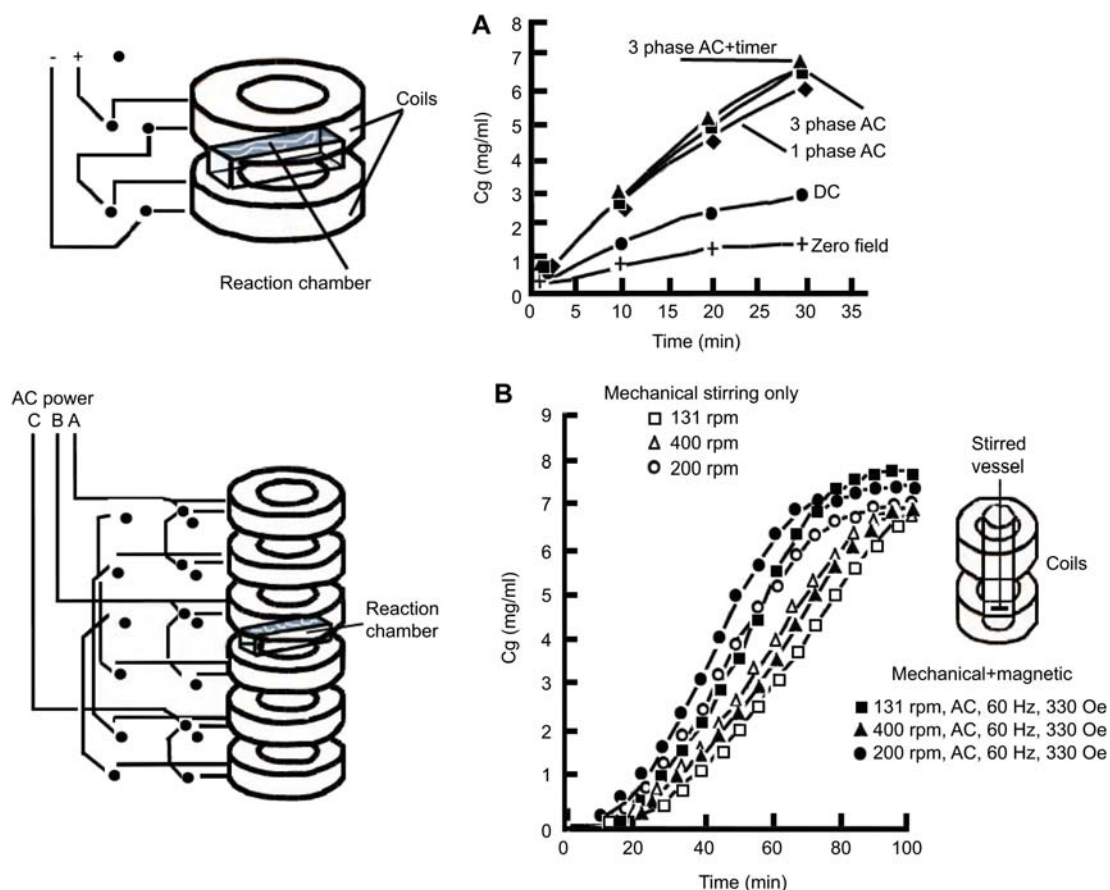


Figure 43 Microreactor with coimmobilized enzymes in chitosan bead. Effect of magnetic field on starch hydrolysis using the chitosan magnetic beads with coimmobilized enzymes. Adapted from Yang K et al. (2010). (A) Hydrolysis in 1.5 ml unstirred vessel. (B) Reaction in 40 ml batch stirred vessel.

7.3.2. Microchip reactors for highly efficient proteolysis

Li Y et al. (2007a) developed a microreactor with a packed bed of magnetic particles proteolysis (Figure 44A). The reactor operates with magnetic microspheres with small size (~ 200 nm in diameter) synthesized and modified with tetraethylorthosilicate; metal ion of copper and enzyme were adsorbed onto the particle surfaces. This is an easily replaceable and regenerable protease microreactor for enzyme immobilization onto the microspheres and then organized by an external magnetic field in a packed bed confined by the channel of the μ -chip. The main advantage of the packed bed microreactor is that the microspheres can be both easily organized in a fixed bed and efficiently removed by the external magnetic field without additional mechanical manipulations. A similar reactor was developed by the same group (Li Y et al. 2007b) by using a capillary (Figure 44C). The trypsin-immobilized MS microspheres were packed into the capillary by placing a magnet directly over the column (Figure 44C) and flowing the trypsin-immobilized MS microspheres into the column with a syringe pump.

The same concept was developed by Liu J et al. (2007) with trypsin-immobilized superparamagnetic microparticles with small size (50 nm). The packed bed was formed by particles initially located on a glass microchip by application

of a strong magnetic field. Then, a suspension of magnetic nanoparticles (3 mg/ml) was injected into the channel for 1 min, leading to a packed bed of 2–3 mm (Figure 44B). It was demonstrated that the operating conditions in such a microreactor were highly reproducible. The on-chip magnetically assisted microreactor utilizing magnetic nanoparticles in a packed bed mode demonstrated several advantages with regard to the traditional used devices for assays:

- The trypsin-immobilized magnetic nanoparticles can easily be separated from the digestion products by using a magnet.
- It is easy to replace the nanoparticles and make the new microreactor. The total time was less than 1 min under the condition of external magnetic field applied.
- The high surface area-to-volume ratio of the magnetic nanospheres packed within the chip ensures a short distance for the substrate to diffuse from solution to the nanostructured particles where trypsin was located.

These microchip reactors employ the operation mode “magnetization FIRST – particle flow LAST” (see the discussion) because it is not possible to handle the microparticles in the desired location by the gravity only unlike the MFAF macroreactors.

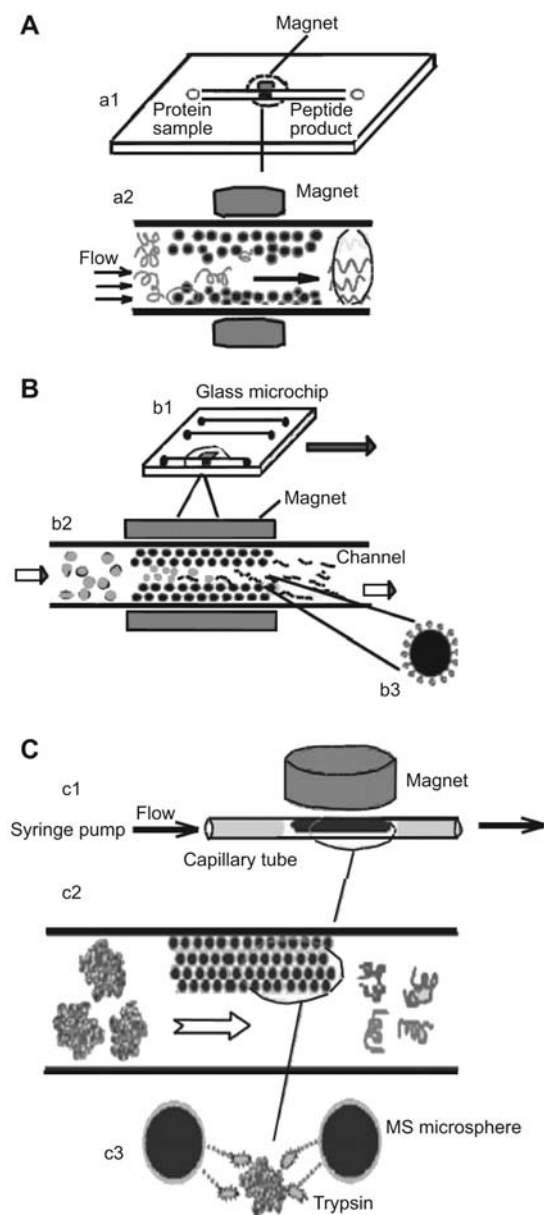


Figure 44 Microchip bioreactors: basic principles in design. (A) From Li Y et al. (2007a). (B) From Liu J et al. (2007). (C) From Li Y et al. (2007b).

Downstream processes

8. Magnetically assisted downstream processes

8.1. Basic concepts

Conventional magnetic separation methods have been used for a long time as standard techniques in a variety of laboratory and industrial applications as the enrichment of low grade ore, desulfurization of coal, etc. Their applications to biochemistry and biotechnology, however, have been restricted and are of limited use up to 1970s according to Safarik and Safarikova (1997). Since then, magnetic separation has

found many useful and interesting applications in bioscience and biotechnology. Generally speaking, there are two types of magnetic separations defined by Safarik and Safarikova (1997, 1999a,b), namely:

- In the first type, material to be separated is intrinsically magnetic so that the magnetic separations can be performed without any modifications. Examples, such as red blood cells containing paramagnetic hemoglobin, MB containing small magnetite particles are only a few in biosciences.
- In the second type, one or more non-magnetic components (commonly diamagnetic ones) or a mixture have to be rendered magnetic by the attachment of a magnetically responsive entity. The new-formed complexes have magnetic properties and can be manipulated by an external magnetic field (Safarik and Safarikova 1997).

MSBs have many advantages over current column separation techniques. The backmixing of solids seen in normal fluidized beds is eliminated, making countercurrent fluid-solid contact possible. Comparable to the fixed beds, the MSBs allow continuous processing of the feed solution at low-pressure drop. Continuous affinity chromatography (Burns and Graves 1987), protein separation (Lochmuller and Wigman 1987, Chetty and Burns 1991) and cell debris filtration (Teranova and Burns 1989, 1991, Al-Qodah and Al-Shannag 2007) were carried out with MSB. The basic results will be discussed below.

8.2. Particles for separations

The functionalized magnetic beads allow quick and efficient purification from crude cell extracts or from other samples rich in cell debris, thereby eliminating the need for most of the pretreatment steps, including centrifugation, filtration and membrane separation (Corchero and Villaverde 2009, Tuzmen et al. 2010). The development of magnetic separations was hindered by a lack of economically viable functionalized supports available for use in bioseparators. Thus, there is a demand for low-cost, robust and reusable magnetic adsorbents (Willett 2009). The magnetic biocarriers were discussed at large in section 4 and the consequent analyses. Now we turn to particles for separation of bioproducts, which in fact, are specific adsorbents used in separations of colloids replacing coagulants by magnetically responsive supports. This concept was firstly developed in the high gradient magnetic fishing method (Hubbuck and Thomas 2002) demonstrating better versatility than the commonly used expanded bed adsorption technique (Willett 2009).

The use of magnetic particles for separations avoid the problems emerging in the application of porous adsorbents because no intraparticle diffusion and binding are required to attain the desired separation. Therefore, the absence of pores also avoids the fouling of the adsorbents. Because only surface adsorption works in the magnetic non-porous support, their contact area is lower than that of the classical adsorbents. However, this is not a disadvantage, because by incorporation of magnetic particles into the non-magnetic

bioactive adsorbent materials, it is possible to attach specific proteins, for example, from the bulk of the liquid where the supports are immersed.

Because many materials (polymers) are available as potential biosupports for separations of proteins and cells from liquids, the key problem is how to make them magnetic and selective. The magnetic compounds were discussed in the case of biocarriers (section 4). Generally, there are differences in preparation in beads for both purposes – immobilization and separations. However, in the former case the solids should be porous, thus allowing large surface and volume for immobilizations of cell and enzymes. In contrast, the particles for separations have to develop only active to adsorption surfaces and the intraparticle diffusion has to be minimized.

There are many methods to make the magnetic particle functionalized. The most common ones are the dye-affinity adsorbents, for example, even though other approaches are available in the literature. Dye-affinity adsorbents for protein purification allow simple, cheap and rapid isolation of proteins, typically with 20- to 80-fold purification, with approximately 80% recovery from a crude extract without prior treatment (He et al. 2007, Tuzmen et al. 2010). The dye immobilization does not use toxic substances. The dyes are all synthetic in nature but they are pseudospecific affinity ligands because they interact with the active sites of many proteins and enzymes. The specific interactions between dye ligands and proteins can be a complex combination of secondary forces including electrostatic, hydrophobic and hydrogen binding (Zhang and Sun 2001).

We do not try to encompass all methods and types of particles available, the dye-affinity adsorbents serve only as an example. To complete this introductory but very important section, some examples of magnetic affinity adsorbents are given, as follows.

A) Protein adsorptions

- Matrix containing magnetite colloids (Xue and Sun 2002) for protein adsorption.
- Ni-Fe-B alloy-densified agarose gel for protein adsorption (Tong and Sun 2001).
- Magnetic macroporous cellulose cation exchangers for lysozyme recovery (Safarik et al. 2007).
- Capture of lactoferrin from whey using magnetic micro-ion exchangers (Meyer et al. 2007) and superparamagnetic polyglycidyl methacrylate particles coupled with heparin (Chen L et al. 2007).
- Adsorption of bovine serum albumin on nanosized magnetic particles (Peng et al. 2004).
- Chelating group iminodiacetic acid coupled to the surface of magnetic agarose and charged with metal ions (Cu^{2+} or Zn^{2+}) for binding proteins which display metal affinity (Abudiyab and Beitle 1998).
- Cibacron Blue F3GA is commonly used through covalent immobilization to magnetic polymeric beads to remove:
 - albumin from human serum (Altıntas and Adil Denizli 2006),
 - dye ligand affinity chromatography: β -casein adsorption (Tuzmen et al. 2010).
- Phospholipid-coated particles for protein separation (Bucak et al. 2003).
- Magnetic ovalbumin and egg white aggregates for lectins separation (Sabatkova et al. 2008).
- Purification of *Solanum tuberosum* tuber lectin using magnetic chitosan particles (Safarikova and Safarik 2000).

B) Bacteria capture and detection

- Vancomycin combined with FePt magnetic nanoparticles allows a sensitive and quick assay to allow Gram-negative bacteria such as *E. coli* to be captured and analyzed (Gu et al. 2003a,b).

C) Enzyme removal

- Alginate microparticles as affinity adsorbent for removal of α -amylases (Safarikova et al. 2003).
- Covalently binding polyacrylic acid on Fe_3O_4 superparamagnetic nanoparticles for the recovery of lysozyme (Liao and Chen 2002).
- Phenylalanine containing hydrophobic adsorbent for selective and efficient separation of lysozyme from chicken egg white (Uygun et al. 2009).
- By magnetic crosslinked erythrocytes for batch isolation and removal of various proteolytic enzymes (Safarik and Safarikova 2001).
- Non-porous bacitracin linked magnetic adsorbents for *in situ* removal of proteases secreted by *Bacillus licheniformis* (Kappler et al. 2009).
- Purification and characterization of the lysozyme (Kopacek et al. 1999).

D) Other examples

- Thiophilic magnetic beads for purification of antibodies from human serum (Qian et al. 2010).
- Bilirubin removal from human plasma with albumin immobilized magnetic beads (Rad et al. 2003).

8.3. MSB-based protein separations

8.3.1. Affinity chromatography by a moving admixture

MSB The only systematic studies on protein adsorption in MSB devices for affinity chromatography were performed by Burns and colleagues (Burns et al. 1985, Burns and Graves 1987, Chetty and Burns 1991). Burns conceived a continuous contacting system with adsorption-desorption processing in MSB (Figure 45A) (Burns and Graves 1987). The magnetically susceptible solids are introduced at the top of the first MSB. The feed mixture containing the product is injected at the bottom of the bed. The desired product adsorbs on the support with the non-adsorbed components that travel up with the solvent. The solids are continuously removed from the bottom of the column and transported to a second stage with MSB for a washing procedure. Finally, the magnetic beads are moved to the final MSB fluidized with a desorbing solution. The desorbing product is collected

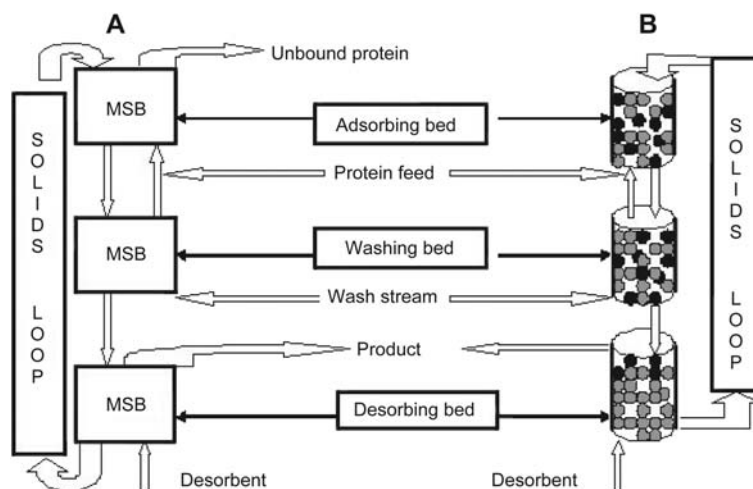


Figure 45 A continuous protein recovery using MSB according to Burns and Graves (1987). The desired protein adsorbs on the solids support in the top bed and is carried downward through the washing bed and collected in the desorbing bed.

while the solids are treated and recycled to the first adsorption column.

The above process has been applied to the particular case of affinity chromatography. Affinity chromatography by an affinity ligand attached on a magnetic support could be considered as an adsorption-desorption process. Detailed mathematical analysis of the system was performed by Burns and Graves (1985). Several simplifying assumptions allowed finding a closed-form analytical solution, including:

- Liquid and solids flows were considered to be in plug flow.
- Adsorption and desorption rate were assumed to be proportional to the protein in both phases. This assumption produces two constants K_a and K_d representing the contributions of the protein transport to the surface and the reaction with the ligand, respectively.
- Both the diffusion and dispersion in the axial direction are negligible and all the solute movement is due to convection. This is a rather strong and unrealistic simplification (see Part 7 for detailed analysis of the axial and radial dispersions in MSB).

The analysis performed led to two parameters describing column operation:

$$\alpha = K_a \frac{L}{V}, K = \frac{V}{W} \frac{K_d}{K_a} \quad (19)$$

If α is large and $K < 1$, then the adsorption will occur within a very short distance from the bottom of the first bed (Figure 45). A small value of α implies that a long column is needed to assure the desired solute/support contact time.

Under these assumptions, human serum albumin was adsorbed on Cibacron Blue F3GA, an affinity ligand attached on especially designed Ca-alginate/magnetite beads (Burns et al. 1985). The experimental system is illustrated in Figure 46. The experiments were performed in a Plexiglas column (12.7 mm ID and 100 mm in height) assisted by an axial magnetic of a Helmholtz pair (field intensity of approximately 40 Oe). Flow rates of approximately 10 ml/min for the liquid and 0.5 g/min for the beads were used for a 5-cm high bed (residence times of 30 s for the liquid and 10 min for the solids). The results of several experiments are shown in Figures 47 and 48.

The experiments of Burns reveal that the changes in the feed concentration have little effect on protein adsorption (Figures 47 and 48). The effect of bed height is on the increase in the contact time (the increase in bed height from

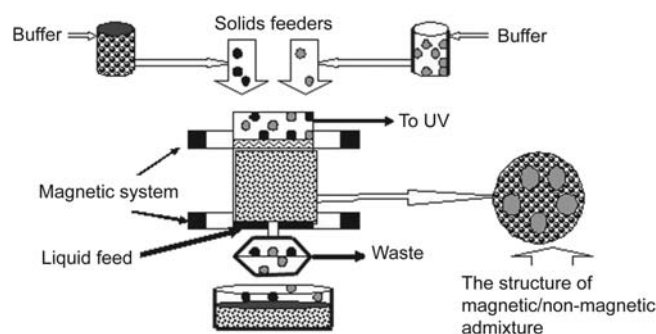


Figure 46 Detailed schematic diagram of the adsorption system used in the experiments of Burns and Graves (1987). Note: black circles – magnetic particles; gray circles – non-magnetic adsorbent. Adapted from Burns and Graves (1987).

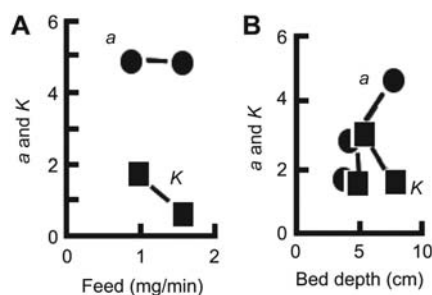


Figure 47 Experimental data of Burns and Graves (1987) (data extracted from tables 1 and 2 in original article). (A) Effect of liquid feed on a and the dissociation constant K . (B) Effect of bed height on a and the dissociation constant K .

5 to 8 cm leads to corresponding values of α 3 and 4.8, respectively). Burns and Graves (1987) pointed out that the adsorption is sensitive to the dissociation constant K and relatively insensitive to the rate constant α (Figures 47 and 48).

The affinity adsorption discussed above employed supports specially designed to be both capable of binding affinity ligand and magnetically susceptible (Burns et al. 1985). The lack of such commercially available magnetic supports (in the time of the investigations) necessitates the development of such supports for each specific application. Because of this, the further investigations of Burns were directed to the problem with the choice of protein adsorption of stabilized bed of admixtures of magnetic and non-magnetic particles (ion exchange resins) (Chetty and Burns 1991) (Figure 45B). The external field allows the magnetic particles to form a matrix entrapping the non-magnetic supports: the magnetic ones stabilize the bed, whereas the non-magnetic beads are used to adsorb the desired biochemical. Such MSBs have no restrictions of having to use adsorbents that are magnetically susceptible. The hydrodynamic behavior of gas-solid and liquid-fluidized MSB of admixtures were reviewed in Part 1 and Part 5, respectively. The experimental set-up is illustrated in Figure 46 (the inset schematically shows the bed structure).

Amberlite IR-120 (500 μm) and Macrosorb.KAX.CB (100–300 μm) were stabilized by nickel particles

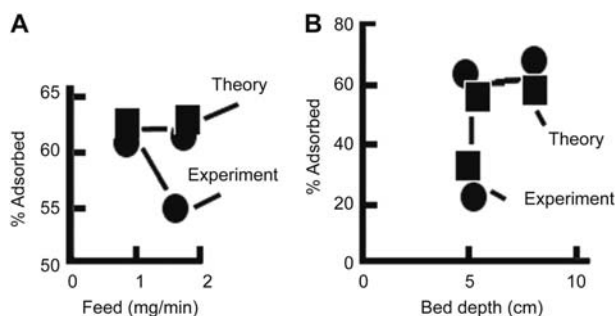


Figure 48 Experimental data of Burns and Graves (1987) (data extracted from tables 1 and 2 in original article). (A) Effect of liquid feed on the amount of the adsorbed protein. (B) Effect of bed height on the amount of the adsorbed protein.

(100–500 μm) in two columns (2 cm ID and 7.5 ID). The bed height was varied from 3 and 5 cm (in 2 cm ID) to 7 and 9 cm (in 7.5 ID column). Chicken egg white lysozyme (molecular weight of 14,500) and a myoglobin from horse skeletal muscle (molecular weight of 18,800) were tested as a mixture undergoing separation.

With a field intensity of 75 Gauss and a fluidizing velocity of 6 cm/min MSBs with 10–60% (vol.) of non-magnetic resins are created; the exact amount of non-magnetic solids entrapped by the MSB depends on the type of the non-magnetic support and the operating conditions (see Part 1 and Part 5).

The Macrosorb-lysozyme pair displays a non-linear adsorption isotherm (Langmuir).

$$q^* = \frac{q_m C^*}{K_d + C^*} \quad (20)$$

The maximum capacity of the support has been established to $q_m = 21$ mg/ml. A minimal lysozyme adsorption on nickel (lower 1 mg/ml) has been detected. It was assumed that nickel did not affect the activity of lysozyme.

With the range of bed heights examined (3–9 cm) it was found that between 40% and 85% of the feed protein was adsorbed. Higher bed heights led to higher fractions of protein adsorbed due to the increase residence time of the liquid. By contrast, the increasing solids flow has demonstrated an increase in amount of the protein adsorbed due to the increased driving force for adsorption. In both cases, the higher particle-liquid slip velocity decreases the limiting diffusion mass transfer resistance (Burns and Graves 1985). The same effect was detected as a result of the increase in the rate of addition of the fresh resin. The later decreased the surface concentration of protein on the surface and, therefore, increased the mass transfer rate. The experimental results reveal that the change of solids flow rate from 2 to 8 ml/min augments the fraction of protein adsorbed from 40% to 85% (figure 8 of Chetty and Burns 1991). The increases in the resin fraction of the bed led to increased separator efficiency; but this was valid for the regime of MSB only. The latter simply means that 100% non-magnetic bed became fluidized at a velocity of 6 cm/min and exhibited an adsorbing capability equivalent to a MSB with 35% affinity supports.

Special experiments were performed with a mixture of lysozyme (0.3 mg/l) and myoglobin (0.3 mg/l). The experimental conditions were similar to those in the lysozyme adsorption experiments. A bed of 9 cm (25% Macrosorb and 75% nickel spheres) adsorbed approximately 80% of the feed lysozyme. However, approximately 10% of the feed myoglobin was adsorbed (6% on the resin and 4% on the nickel) (figure 12 of Chetty and Burns 1991; see also Goetz and Graves 1991).

8.3.2. Expanded bed-like approach (MSB regime)

Unlike the affinity chromatography of Burns and Graves, operating in a magnetically stabilized moving bed of admixtures, the expended bed adsorption, a well-known technique, was tested by several authors, including:

- Noble et al. (1989) conceived a MSB-based separator of proteins employing a Helmholtz pair and a lysate entering the column at the bottom. The results (the examples in the patent) claim that with increase in the voidage of the MSB the cells contained in the lysate will pass through the bed without forming clogging, i.e., no fouling and plugging in the bed. The proteins will be adsorbed on the magnetic ion-exchange particles forming the bed. No examples of the adsorption performance of the bed exist in the patent description.
- Zhang Z et al. (1999) used low-density composite magnetite-agarose adsorbents (1120 kg/m^3) that were fabricated by a bead emulsification process following derivatization with a peptide for the purification of a recombinant protein from *E. coli*. The stabilization was performed in a high intensity wet magnetic separator (type LHWL, Boxing-Rapid, Ltd) with field transverse to the fluid flow. The field was established at 0.1 T. The MSB of such beads was firmly controlled when the fluidizing medium was *E. coli* broth (up to 20% wet/v/v). Zhang Z et al. (1999) mentioned channeling in the bed structure, especially in the highest yeast concentrations. The negative effect of the channeling was compensated by recirculation of feedstock through the bed and thus extending the residence time.

A comparative analysis between MSB and a non-magnetic version (at zero magnetic fields) resulted in the conclusion that an unacceptable high proportion of particles from the column was entrained in the flow. It was possible to recover those particles in MSB because a recirculation of the broth was performed. Finally, the authors decided that the most amenable as a physical alternative was a non-magnetic bed with higher density. To this end, it is worth noting that this conclusion is a result of the incorrect performance of the experiments and some principle reasons, including:

1. A magnetic separator which was not designed for magnetic stabilizations was used. Such devices have strong lateral gradients of the field transversally to the fluid flow because their main purpose is to capture particles and attach them to the poles. If the field is homogeneous, magnetic pulling forces do not exist and there is no magnetic separation. Hence, the lateral non-homogeneity is inherent for the magnetic field of the electromagnet used.
2. MSBs need homogeneous fields because a homogeneous fluid-solid contact is needed. This is the main condition, for example, in the classical expanded bed adsorption columns. If the field is strong enough, transversal to the fluid flow, then the particles form a clump at the column wall and the central zone starts to work as a channel because there are no particles there. Hence, the basic principle to create a fluid-solid contactor was violated.
3. This study is a good example of the principle: Let us see what it would be if a bed and a given magnetic system available in the laboratory will be combined. The outcome is always negative, because the preliminary thinking was avoided. However, the most influencing outcome is that the published results form a basis for a general

opinion that the MSB absorber does not work well. Actually, the experiment was badly designed.

Tong and Sun (2003) conceived a magnetite-agarose support (MAS) for protein adsorption in a liquid-solid MSB. A reactive triazine dye, Cibacron blue 3GA (CB), was coupled to the gel to prepare a CB-modified magnetic agarose support (CB-MAS) for protein adsorption. Lysozyme was used as a model protein to test the adsorption equilibrium and kinetic behavior of the CB-MAS. A transverse homogeneous magnetic field was created by two poles (Figure 49) (Tong and Sun 2003). The operating mode was magnetization FIRST. Residence time distribution experiments and axial dispersion coefficient estimation were performed *lege artis*. The work of Tong and Sun (2003) is a very good example how a study and the consequent scientific report on mass transfer in magnetically assisted beds should be done.

Tong and Sun (2003) investigated the breakthrough curves of lysozyme in a packed bed (PB), expanded bed (EB) and MSB under identical operating conditions. The low voidage in the PB column leads to a steep breakthrough curve unlike those exhibited by the expanded EB and MSB. The dynamic binding capacities of lysozyme for the three separating devices are shown in Figure 49B. The results reveal that the efficiency of lysozyme adsorption in the magnetically stabilized fluidized bed is much better than that obtained in the EB and similar to or even slightly better than that in the PB, a fact just opposite to that derived from the improper experiments of Zhang Z et al. (1999). The parameters of the Langmuir isotherm are shown also in Figure 49B. The better performance of the MSB adsorber is in agreement with the axial dispersion data (Figure 49C). Note, Part 7 revised all existing studies on axial dispersions in MFAF. In the context of protein adsorption by magnetic beads, the results and model developed by Xue and Sun (2001, 2002) as well as Tong and Sun (2003, 2005) are highly recommended as templates of well done studies and sources of information.

The key problem in protein recovery by the MSB is the particle design. All specific problems of particle surface functionalization cannot be encompassed, which, in fact, is beyond the scope of the present review, but the analyses of Safarik and Safarikova (1999a,b, 2004) about particle surface modifications is recommended as well as Aasim et al. (2010) concerning the surface energetics of protein adsorption onto a support among the further readings prior to some concepts about studies in this direction.

8.4. MSB-based cell affinity separations

The separation of specific cells from mixed populations is essential for a complete understanding of the role of these cells in complex, normal and pathological processes. Several attempts, discussed next, were performed to use magnetically assisted beds to separate cells. The principle of the immunomagnetic cell separation (sorting) is explained by the inset in Figure 50 (after Thiel et al. 1998).

Molday and Molday (1984) were pioneers in the field by using iron-dextran microparticles and high-gradient separator. The dextran was conjugated to Protein A to allow a

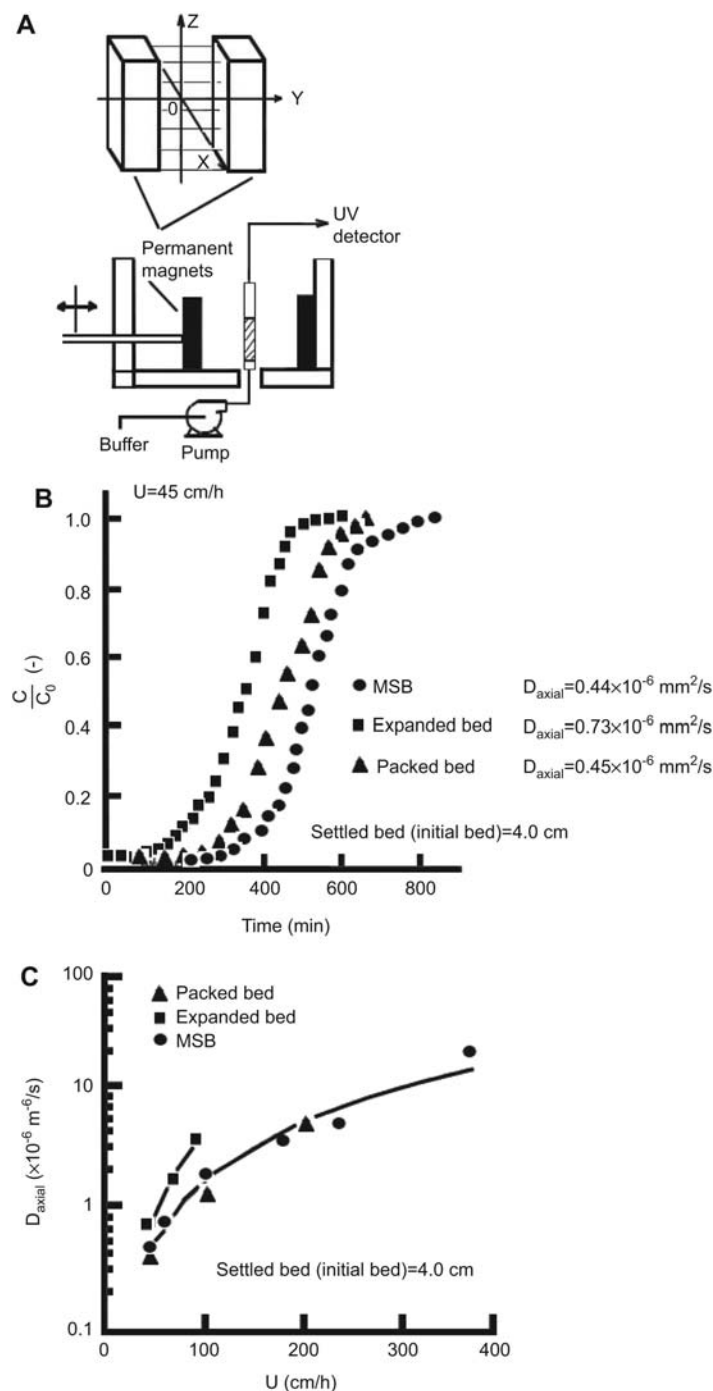


Figure 49 Experiments of Tong and Sun (2003) on protein adsorption in MSB. (A) Experimental set-up (schematically). (B) Effect of the fluid velocity on the axial dispersion in three beds of the same particles but with different structure (particle arrangement). (C) Breakthrough curves for three beds with different particle packing.

specific label of red blood cells (RBCs). A column containing microparticles and stainless steel wires was exposed to a 7500 Gauss magnetic field created by the flat poles of an electromagnet (Figure 50A): a 2-cm gap was used. When 10^7 RBCs sensitized with rabbit antihuman RBC antibodies and subsequently labeled with Protein A-ferromagnetic iron dextran conjugates were applied to a column packed with

30 mg stainless steel wire and placed in an applied magnetic field of 0.75 T, over 96% of the cells were retained on the column. Most of the remaining cells could be eluted after mechanically agitating the column on a vortex mixer. The total recovery of cells applied to the column was 98%. The field intensity effect on the capturing efficiency is shown in Figure 50B. Molday and Molday (1984) investigated the

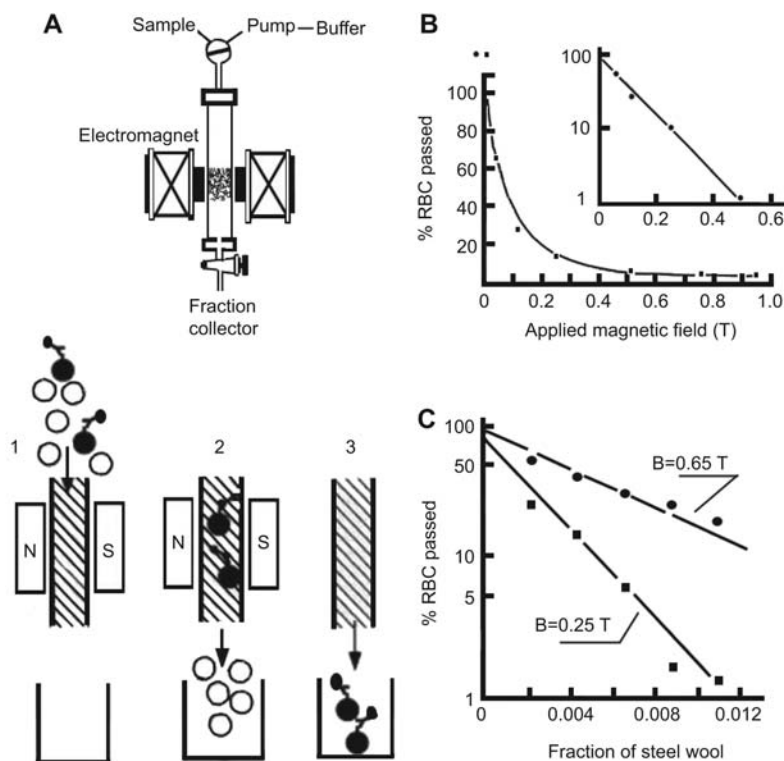


Figure 50 Experiments of Molday and Molday (1984) on immunomagnetic separation of red blood cells (RBCs). (A) Equipment – schematically (Molday and Molday 1984). (B) Field effect on the passage of RBCs labeled with immunospecific iron dextran; 30 mg iron steel wool in the column. Inset: logarithmic plot. (C) Effect of the amount of magnetic mass (iron steel wool) on the separation efficiency. Inset: principle of the immunomagnetic affinity separation. Adapted from Thiel et al. (1998).

effect of the magnetic fillings of the column (steel wire) on the percentage of cells which passed through the column at constant applied field and constant flow rate (Figure 50C).

In fact, the study of Molday and Molday (1984) did not use a MSB in its true version. More precisely, the approach used resembles that of screened MSBs (see Part 2) where the particles adhere magnetically on bars or wires with stronger magnetic properties. This approach allows to “immobilize” easily by magnetic forces small amounts of particles and a large free volume of the bed to be attained.

Chalmers et al. (1998) developed the concept of cell sorting by creating a continuous flow-through immunomagnetic cell separation (Figure 51A,B). The main concept is a flow of labeled cells through a dipole or a quadrupole magnetic system (Figure 51B) creating strong magnetic gradients. The dipole design can “fractionate” an inlet, magnetically labeled, cell stream into different outlet streams on the basis of the degree to which the cell is immunomagnetically labeled. Otherwise, the quadrupole separator splits an inlet immunomagnetically labeled cell stream into two outlet streams. The purity, recovery and potentially the degree to which the cells are immunomagnetically labeled is controlled by the flow rates in the inlet and outlet flows. A 99% purity and 86% recovery were achieved with the quadrupole system (Chalmers et al. 1998).

The concept of cell affinity separation on functionalized particles in the MSB was really demonstrated experimentally

by the group of Burns (Putnam and Burns 1997, Putnam et al. 2003). Binding specificity of the magnetic beds was obtained by immobilizing the lectin *Helix pomatia* Agglutinin (HpA) or *Griffonia simplicifolia* I (GSI) onto a magnetite-containing support. The experimental set-up used an axial magnetic system created by four coils equidistantly placed along the column axis (Figure 52A). Separation of type A

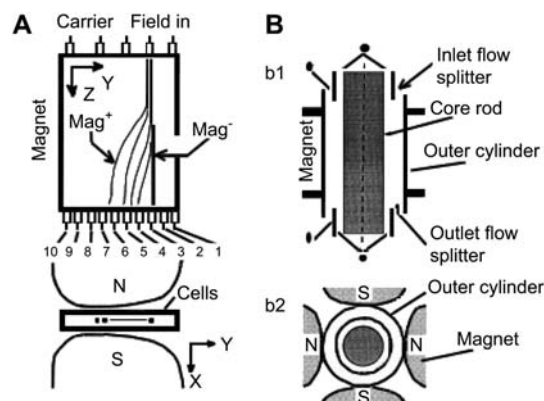


Figure 51 Cell affinity separation in high gradient magnetic field (HGMF). Adapted from Chalmers et al. (1998). (A) Principle of separation in HGMF in a dipole separator. (Top) side view, (bottom) top view. (B) Schematic diagram of quadrupole HGMF separator. (b1) side view, (b2) top view.

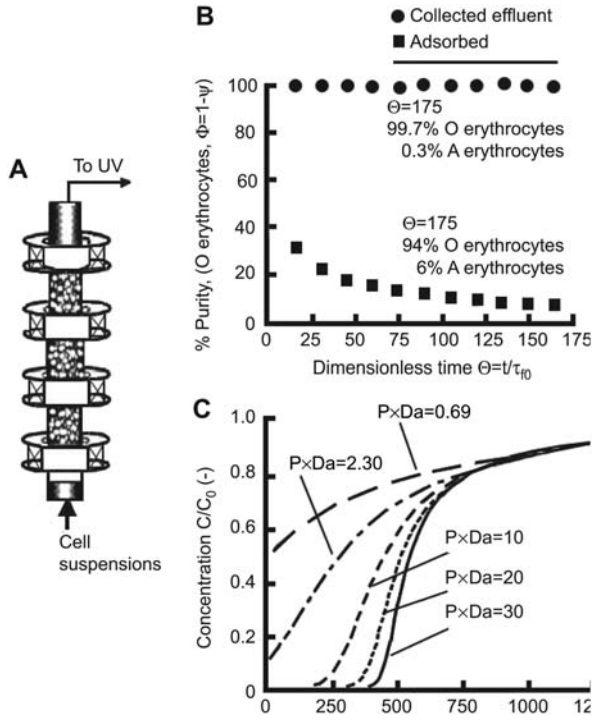


Figure 52 Cell affinity experiments of Putnam et al. (2003). (A) Experimental set-up (schematically). (B) Separation of A and O erythrocytes with *Helix pomatia* agglutinin (HpA). Total number of cells processed is 1.1×10^9 . Adsorbed A (O) cell purities were calculated from the effluent concentration profiles for each cell population. Adapted from Putnam et al. (2003). (C) Effect of adhesion efficiency (P) and Damkoehler number (Da) on breakthrough behavior. Adapted from Putnam et al. (2003).

and type O erythrocytes with the lectin HpA was particularly effective leading to 94% purity of the retained type A erythrocytes (Figure 52B).

The maximum target cell retention occurring during the separation runs is defined solely by the Damkoehler number Da and the relationship of the efficiency was expressed as:

$$f_{sep} = 1 - \exp(-Da) \quad (21)$$

This is a classical filtration efficiency equation, commonly expressed as $f_{sep} = 1 - \exp(-\lambda_f L_f)$ where L_f is the depth of the filter beds and λ_f (m^{-1}) is the filtration coefficient. The Damkoehler number was defined as:

$$Da \equiv 3L_f(1-\varepsilon)^{\frac{1}{3}} \left(\frac{\eta_0}{2d_p} \right) \quad (22)$$

thus relating the bed depth L_f , the particle diameter d_p and the initial collection efficiency η_0 . This definition of the Damkoehler number shows that its value increases with either an increase in the bed depth at fixed particle diameter or a decrease in the particle diameter (at fixed bed depth).

The separation efficiency Φ (Figure 52B) and the target cell population bound $\Psi(\Theta)$ are defined as:

$$\Phi = \frac{\text{contaminating cell in effluent}}{\text{total cells collected in effluent}} \quad (23)$$

$$\Psi(\Theta) = \frac{\text{number of target cells bound}}{\text{total of cells bound}} = \frac{1}{k} \left(\Theta - \int_0^\Theta \frac{C}{C_0} d\Theta \right) \quad (24)$$

$$k = \frac{\text{maximum number cells bound (monolayer) on solids}}{\text{maximum number cells in one liquid void volume}} \quad (25)$$

The experiments of Putnam et al. (2003) show the effects on the cell retention in the MSB (Figure 52C) represented by the breakthrough curves. The dimensionless time is defined as $\Theta = t/t_{f0}$, where $t_{f0} = L_f/U$ exactly matching the hydraulic residence time (U) is the superficial fluid velocity.

The adhesion efficiency P in a product with Da shows the combined effect of the unit cell collector efficiency and the total reactor performance in time. The adhesion efficiency depends on the angular position (φ) of the particle approaching the grain, the critical shear rate (SR_{crit}) and the superficial fluid velocity U , namely:

$$P = \sin^2(\varphi) \quad (26)$$

$$P = SR_{crit} \frac{d_p}{3UA_H} \quad (27)$$

A_H is the Happel parameter depending on the porosity ε through the parameter $\gamma = (1-\varepsilon)^{1/3}$, namely:

$$A_H = \frac{(2-\gamma^5)}{2-3\gamma+3\gamma^5-2\gamma^6} \quad (28)$$

8.5. Cell debris separation

The unique feature of the MSB to prevent particle mixing has been employed for cell debris filtration. The concept of a continuous separator (Figure 53) was developed for a deep bed filtration with the MSB as a filter medium (Terranova and Burns 1989). To some extent, this resembles the concept of the process organization in the cell affinity separation but the major difference is: the particles used for cell debris separations are not surface functionalized. They are simple magnetic particles and the MSB works as a magnetically controlled deep bed filter (Hristov 2007b, Hristov and Fachikov 2007, Abbasov et al. 2010).

The experimental set-up of Terranova and Burns (1989) is schematized in Figure 52B. Counter-current contact between the magnetizable bead and the separated cell debris was created. Fresh cell debris of *S. cerevisiae* yeasts were separated from a liquid flow containing 0.5 g/l glucose at pH 6.5.

The experiments have been performed in a column (2 cm ID column) arranged with a bed of nickel particles

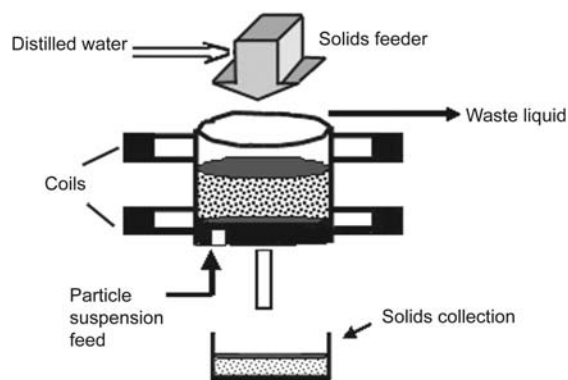


Figure 53 Cell separation in MSB. Adapted from Terranova and Burns (1989). Experimental set-up (schematically).

(100–150 μm) without preliminary surface treatment. The magnetic field generated by a Helmholtz pair has been applied at a fixed intensity of 56 Gauss. The experimental runs were focused on liquid feed velocity (up to 34 cm/min) effect on the separation efficiency. Parallel experiments have been performed with resuspended cells (suspended in a broth). The main results can be outlined as follows:

- The electrostatic interaction between the negatively charged debris and the positively charged support is the primary collection mechanism of yeast cell debris in the bed.
- The change of the liquid feed velocity from 2 to 6 cm/min increases the amount of the debris removed from approximately 10% to 40% (data from figures 3 and 4 of Terranova and Burns 1989).
- The increase in ion concentration (magnesium and sodium ions) from 10^{-5} M to 10^{-1} M increased the fraction of debris collected (figure 5 in of Terranova and Burns 1989).
- The decrease of pH from 6.5 to 4.8 increased the fraction of debris removed with 20% within the range of support liquid velocity from 1.5 to 6.5 cm/min.
- The MSB has a greater capacity for cells than for debris (approximately 5 times) that can be attributed to the smaller size of debris.
- Because typical fermentation broths are 0.01–0.05 dry wt% solids (Alba et al. 1973), the experiments (0.03% wt) of Terranova and Burns show that the device with the MSB is capable of handling the bioprocessing effluent streams.
- Moreover, the filtration device does not produce a cake as other conventional devices (plate or frame filters) thus the clarified liquid could be sent to the units for solute removal.

Al-Qodah and Al-Shannag (2007) did attempt to perform cell debris filtration in an MSB created in a transverse magnetic field. The bed operates in a batch-solids mode (see Part 1 and further in this review). The results are traditional for MSB filtration (see Putnam and Burns 1997, Hristov 2007b) and show that creation expanded bed structure with increases in the magnetic field intensity and transversally oriented field

lines: it is possible to create an effective filter for cell debris filtration. Unfortunately there is no deep analysis and relationships created in this study that would allow establishing some relationships between the operating variables and the filtration efficiency unlike the studies of Putnam (Putnam and Burns 1997, Putnam et al. 2003).

9. Closing comments

Part 8 of this review series devoted to magnetically assisted bioprocesses is almost completed. It was a difficult task to collate problems and viewpoints which never appeared in a common overview. The free-cell reactors, for instance, develop themselves as a separate branch without any relationships to the processes with magnetic biocarriers. However, as was shown in this review, the common problems are in several directions, including:

- Development of basic knowledge prior to application of magnetic fields to microorganisms in proliferation studies or involved in bioprocesses.
- Correct design of both the reactors and the magnetic systems. In this context, the current status of MFAF offers suitable magnetic systems and reactors which could be successfully applied to free-cell processes.
- There is a gap between the proliferation studies and those concerning fermentations under submerged conditions (free-cell processes) or with biocarriers.
- In some studies, the field application is a magic tool, especially those concerning fermentations. The analysis provides that there are no adequate designs of both the reactors and the magnetization units developed so far.

The magnetic biocarriers are on the top of a “tsunami” of publications but little can be found about application to real bioprocesses involving immobilized enzymes or cells and even encouraging laboratory experiments thereof. The current situation is a result of several factors, including:

- The “boom” of research in material science without next steps towards applications. Most of the articles on magnetic supports stop at the points of bead characterizations.
- The people working on magnetic biocarriers applications, in general, apply them in a conventional manner as free floating supports that can be easily removed by conventional magnetic separators. However, such applications face the same problems as those with conventional biocarriers: low slip velocity, problems with the density ratio, limited fluid flow (dilution) rate, etc.
- Missing collaborations at the edge of reactor design, magnetic biocarriers (both preparation and applications) and adequate magnetic systems. Obviously, this envisages a broad viewpoint by the researchers, which can be compensated either by creation of effective interdisciplinary teams or more ample knowledge outside the narrow specialization.

In any case, in fact, that the present review encompassing research for around 25 years will provide sufficient infor-

mation to the researchers involved in magnetic field applications to bioprocesses and, the most important issue, will provide the level beyond which the next research should be done. It was a difficult task but also a pleasure to create this review and make it available to all people interested in this topic.

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