Microbial resources and industrial microbial processes design and behavior

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The large diversity in the microorganisms explain why they are involved in many industrial microbial processes. The industrial microbial processes concern fermented food preparations, microbial cell and metabolite productions and wastes processing. They are made in fundamentally the same way with three steps: pretreatment of raw matter, microbial conversion, and product recovery. The obtaining an efficient process is based on the control of the environmental conditions and the limiting substrate concentration influencing the microbial metabolism. Microbial process design should take into account specific biological and technological constraints and minimize the cost of product during production and downstream operations.

Keywords: biodiversity; bioreactor; microbial process; fermented food; metabolite; waste.

1. Introduction

A large number of natural ecosystems are sites of impressive diversity of microbial activities. Bioprocess engineering evolves from the use of biocatalyst activities especially microorganisms by scientists and engineers in order to obtain products or services [1, 2]. The industrial microbial processes (IMP) resulted in the control of safety, mixing and environmental conditions and in the use of selected microorganisms to obtain higher productivity with lower cost. They could contribute also to solve the problem of increased demand in the international market in natural products with good safety with respect to health and environmental restrictions.

Innovative research is therefore urgent in order to establish technologies economically viable which operate on the spectrum of opportunities offered by microorganisms. From the gene to the production system, the bioengineering is under constraint of ecoethics, sustainability and public acceptance.

The multi disciplinarily of bioengineers is related to the main rule of the optimization of the bottlenecks and the elimination of the extra and intracellular key process constraints. Microbial engineer includes the "omics" (transcriptome, proteome, metabolome, physiolome) under the effect microbial populations, connected to macro, micro mixing and biological reactivities. The education of microbial engineering drives scientists and practitioners to conduct microbial processes with innovative mind and high productivity.

This paper present the integrated knowledge of microbial resources and bioengineering necessary to the faster development cycle of industrial microbial process and the generation of the appropriate manpower for society.

2. Bioengineering of diversified microbial metabolism

Microbial activities induce the microorganisms as dynamic entities and continually changing by transformation of essential and indispensable chemical elements assimilated from environment into

constituents of which cell is composed. Schematically the catabolism produces energy by oxidation of catabolite substrate while the anabolic pathway built from substrates just the monomers, secondary biopolymers constituting the cell biomass (Fig. 1).

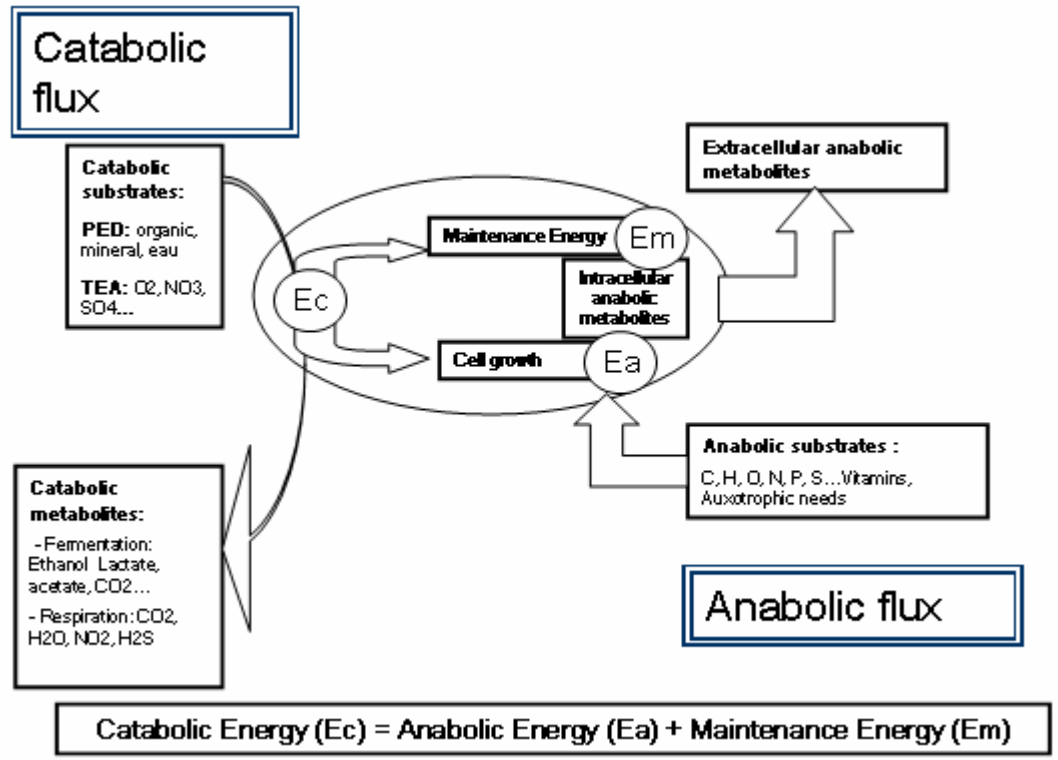

Fig. 1. Mapping of the different barriers flux and actions controlling the genome expression source of bioproducts in industrial microbial processes.

The change of energy and carbon sources used gives a large diversity in the microorganisms and explain why they were presents during biosphere evolution and involved in many industrial microbial processes and ecosystems [5, 6]. The oxidation of the catabolite substrate which is an electron donor requires some electron acceptors according microbial groups (Table 1). The synthesis of the high energy phosphate bonds in ATP resulted from the series of reactions involved in the oxidation of primary electron donor (PED) and the reduction of terminal electron acceptor (TEA). Energy is trapped largely as ATP either during oxidation at PED level, photophosphorylations or in oxidative phosphorylations which occur as hydrogen from the catabolite substrate is transferred to TEA [7].

Table 1: Microorganism major groups and their energy and carbon source, primary electron donor, and terminal electron acceptor

Energy source	Carbon source	PED	TEA	Microorganisms
Photoheterotrophs:				
Light	Organic C	H_2O	CO_2	Green non sulfur bacteria and Purple non sulfur bacteria
Photoautotrophs:				
Light	CO_2	H_2O	CO_2	Algae and cyanobacteria
Light	CO_2	H_2S	CO_2	Green sulfur bacteria and Purple sulfur bacteria
Chemoautotrophs:				
Mineral	CO_2	H_2S	O_2	Sulfo-oxidizing bacteria
Mineral	CO_2	NH_3	O_2	Nitrifing bacteria
Mineral	CO_2	H_2	O_2	Hydrogen bacteria
Mineral	CO_2	H_2	CO_2	Methanogenic bacteria
Chemoheterotrophs:				
Organic C	Organic C	Organic C	O_2	Fungi, protozoa and most bacteria
Organic C	Organic C	Organic C	Nitrate	Denitrifing bacteria
Organic C	Organic C	Organic C	Sulfate	Reducing sulfate bacteria
Organic C	Organic C	Organic C	Organic C	Some fungi and fermentative bacteria

The level of energy produced and kinds of intermediates which are responsible of coupling between catabolism and anabolism, are function of primary substrates concentrations and catabolic pathways on the one hand, and the biosynthesis pathways of cell constituents and anabolic metabolites on the other hand (Fig. 2). Metabolites produced by microbial metabolism were classified in three products according the coupling of energy-producing reactions with product-forming reactions [8]: (i) catabolic metabolite formation related to carbohydrate utilization, (ii) anabolic metabolite formation indirectly related to carbohydrate utilization during growth limitation, (iii) anabolic metabolite formation not associated with carbohydrate utilization. However at low growth rate, biomass formation becomes energetically more expensive and substrate flux is presumed to solely support maintenance demands [9, 10]. In deed, substrate limitation or stress conditions induce low growth rate because the uncoupling of energy-producing reactions with product-forming reactions.

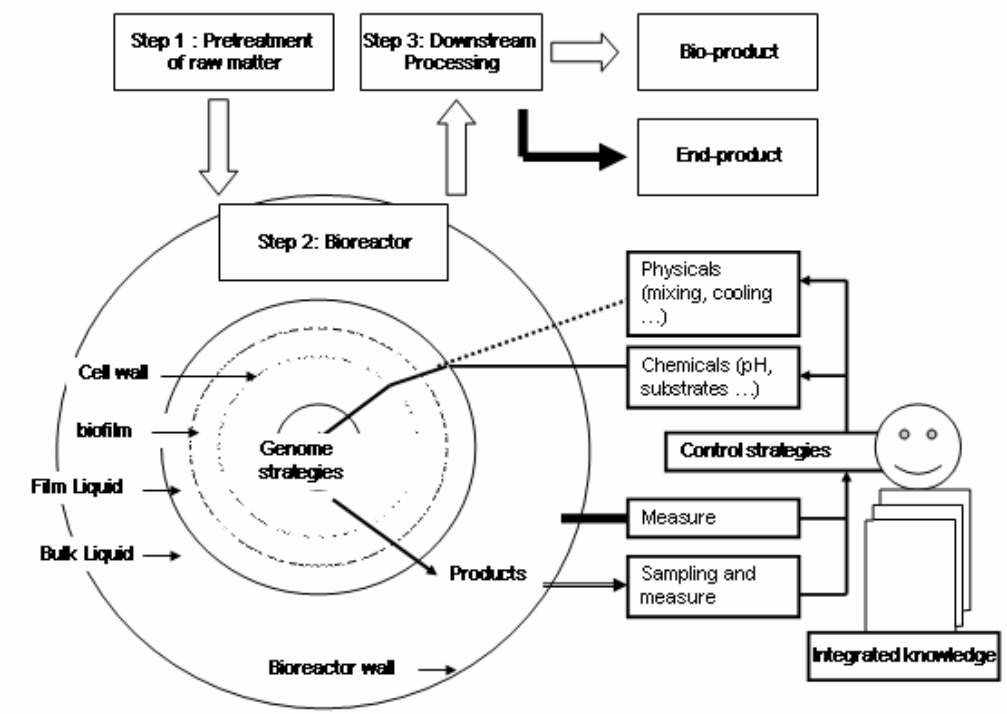

Fig. 2. General scheme of relationship between catabolic reactions and anabolic reactions in microbial and the chemical energy coupling system between dissimilation, synthesis and maintenance.

The control of the microbial metabolism is essential component in the construction of industrial strains and in obtaining an efficient process. Metabolic engineering based on the advent of recombinant DNA technology and pathways control should offer new opportunities in the development of new products and in the improvement of productivity [4, 11, 12].

3. bioreactor behavior and microbial kinetics control

The biochemical reactions involved in the microbial cell growth, the metabolite production and the maintenance are under the control of the environmental conditions and the limiting substrate concentration. However, the microorganisms used in the microbial process are never under optimized conditions but rather under limitations and stressed conditions [11]. The models of consumption rate of limiting substrate used in the microbial cell growth, the metabolites productions and the maintenance are the basis of the development of algorithms for processes and their applications to the control, of critical variables, e.g., temperature, pH and nutrient addition, to optimize productivity [9, 13].

3.1 Environmental conditions

The physico-chemical and mechanical parameters represent possible control environmental conditions in bioreactors. The cultivation of microorganisms requires appropriate culture media containing all chemical needs, and optimal physical conditions as temperature, pH, gaseous atmosphere and mixing.

The extracellular environment is more homogenous than intracellular environment, particularly the cytoplasm's elements change with extracellular environment as osmotic pressure, heat chock and physiological state of organism [14]. In deed, it remain hard work for cell structure in order to modify

some barriers (compartments and fluids) which control the intracellular mass transport and then the biochemical kinetics. The microbial cell freely suspended in a bioreactor often occurs in groups or flocs many orders of magnitude greater in size than a single microorganism. The size and the shape of microbial cells and their aggregation have an important consequence on the transport of nutrients from environment to the inside (Fig. 1). The specific overall rate of substrate uptake is likely to be reduced because of diffusion limitations on the transfer of substrate within the biofloc [15, 16, 17, 18]. The reported diffusion coefficients varied from 7.3 % to 73.6 % of the corresponding value in water and were dependent upon biofilm thickness [19]. The new concept of critical diameter of biofloc D_c [15, 20] assume that from the D_c value, the solid-phase diffusion limitation becomes more significant than the liquid-phase diffusion limitation. When biofloc diameter is greater than critical diameter, the mixing effect on the specific overall rate becomes insignificant and many undesirable consequences on the microbial process can be observed. The mixing time is the most useful criterion for the mixing intensity characterization and for fermentation processes scale up [21].

3.2 Limiting substrate addition methods

The addition of the limiting substrate into the bioreactor in order to control the microbial metabolism (Fig. 2) is done according to three methods of microbial cultures in bioreactors depending the goal of the industrial microbial process (Table 2): batch, fed-batch and continuous culture.
Microbial cultures carried out in batch reactor are characterized by accumulation of all produced cell and metabolites. The batch cultures are used especially in solid state fermentations (SSF) and in fermented foods characterized by successive biochemical reactions.
Fed-batch is the frequently used process in industry and additions of nutriments in bioreactor without outgoing in order to maintain a constant level of substrates are taking place during culture according microbial physiology and fermentation goal [21]. The fed-batch mode of operation is the most-widely used in industrial fermentations for cell and metabolites production. It is suited for microbial cultures that exhibit substrate inhibition, catabolite repression, toxic precursors or the glucose effect [3]. Feed-limiting glucose concentration in the broth avoids the Crabtree effect on a respirometry metabolism for *Saccharmyces cerevisiae* and allows the cells to be grown to high density [22].
Continuous cultures carried out in mixed or in tubular bioreactor with constant volume because are used especially in the wastewater treatment. The residence time which controls the degree of conversion is verified with pure culture but with mixed culture, other phenomenon can change conversion such as competition, synergism, mutualism [8]. With tubular bioreactor, the concentration profiles change, and the degree of conversion is depending of the both residence time and length of bioreactor.
The microbial metabolism is more easily controlled by using fed-batch and continuous methods. The continuous stirred tank reactor (CSTR) should be larger than the batch and the tubular bioreactor in order to accomplish the same degree of conversion [21]. Due to similarity between the autocatalytic microbial propagation and the chemical reaction, it well established that the optimal configuration of continuous operation is the CSTR followed by the tubular reactor. The bioreactor performance can be increased by using the fed-batch or the retention of cells in the continuous bioreactor, especially when the microbial kinetics are not limited by the nature of substrate.

4. Industrial microbial processes

Metabolic activities of microorganisms underlie a variety of industrial microbial process (IMP) ranging from fermented food preparation, to microbial cell and metabolites production to wastes biodegradation, and they have a high impact on most of the food [23, 24, 25], pharmaceutical, chemical industries [26] and on the ecology [27]. Industrial microbiology is becoming an area of innovation and a good example of emerging technologies. All IMP can be classified into three types: food fermentation, microbial cell and metabolites production and waste biodegradation. All equipment used in the IMP must

be validated for a good manufacturing practice (GMP). They are made in fundamentally the same way with three steps: pretreatment of raw matter, microbial conversion, and product recovery (Table 3). Microbial process design should take into account specific biological and technological constraints and minimize the cost of product during production and downstream operations.

The pretreatment step is crucial when raw matter is complex and variable as with fermented food and waste conversion. The choice of suitable operating conditions of microbial process must take into account the physiology and metabolism of industrial selected strains and the goal of the microbial process (Table 2). The choice of microbial process route for industrial application is determined by practical and economical data which must be considered and revised in all steps of development and life of the activity. For all IMP, the control of reproducibility and the quality standard, the optimization of the productivity and the residual substrate are required.

4.1 Fermented foods

Fermented foods are carried out in most parts of the world, and local products are given special names. The production of the fermented foods is based on the microbial activity which induces beneficial changes [28]. The microorganisms involved in the biopreservation of food are especially lactic acid bacteria and some yeast and their essential function is to convert sugars into more stable molecules such as lactic acid and ethanol respectively. Most of the involved microorganisms produce flavor ingredients that give the final product its distinctive taste. The approach to develop or improve a fermented food product, the composition of raw vegetable or animal matter and the required quality of product are determining factors(Fig. 3a). The knowledge of microorganisms present in fermented food is crucial in order define a procedure to control the fermentation and the safety of product. The use of pasteurization of raw matter and dried or frozen starter is the modern way to produce a fermented food at industrial scale. Practically, all fermented food are prepared in batch fermentation because foods are converted by serial microbial reactions and because of the nutritional and safety quality requirements.

4.2 Single cell and metabolites production

The industrial approaches used for production of single cell and metabolites are based on the selection of efficient strains and on the optimization of the culture conditions at laboratory and pilot scale (Fig. 3b). The best way to prepare the scaling-up of process is to first scale-down to the pilot scale of the conditions of cultures that will be used at the final scale of production [29]. Fed-batch fermentation process is widely used for microbial cell and metabolite production [3] and it is ideally suited to use computer control and sensors for measuring important parameters according microbial metabolism related to product formation. The fermentation process is developed taking into account the feedback from the down stream process (DSP) development, the final scale production and good manufacturing practice (GMP) requirements [30].

In fed-batch process, exponential feeding allows the cells to be grown at a constant growth rate which is required for biomass and primary metabolites production. Secondary metabolites which are anabolic products are released in quantity during growth decrease or interruption. The awareness of real scope of microbial genome diversity and growing interest in the biotechnological application of microbial products as pharmaceuticals, bioactives, biocatalysts, biomaterials and so forth must prompt the development of new research techniques for the direct and indirect acquisition of theses genomes [31, 32]. The great need for the future leaps in techniques for isolating and culturing novel microorganisms which can be a valuable resource of gene products and reactions [33, 34]. Synthetic biology involves the creation of artificial gene and metabolic networks to program new cell and organism behaviors [35]. Overproduction of metabolites requires mastery of the fermentation process for each new strain as well as sound engineering know-how for media optimization and the fine-tuning of process conditions [36]. Many bacterial behaviors are regulated by quorum sensing, including symbiosis, antibiotic production and biofilm formation [37].

Table 2: Strategies of design and uses of the industrial microbial processes

Goal of IMP	Industrial Criteria	Biological pathways priorize	Trends and tools
Fermented foods (FF)	Biopreservation of foods Favour the beneficial changes (nutritional and sensory) Improvement of the safety and the hygiene quality	Stimulate the sugar conversion into stable molecules (ethanol and acids) Control of physiology: Production of bacteriocins, the flavours molecules, EPS,... Generally recognized as safe status of microorganisms (GRAS).	HACCP application and ISO certification (GMP, GHP) Standard raw matter of foods Batch culture cultures and uses of suitable starters
Single cell protein (SCP)	Minimize the residual substrate Maximize the yield of cell production. Shelf life of specific activity. Maximize the value of use.	Some vaccine attenuated cells, single cell proteins, starters. Stimulate anabolic pathway. Sometime take care of carbohydrate reserve for shelf life (yeast...).	High growth rate, high dilution rate in continuous. Fed batch or chemostat if Substrate inhibitor. Avoid limitations and stress conditions.
Metabolite production (MP)	Minimize the residual substrate and maximize the product yield. Minimize the cell production. Trend from Microbial growth to bioconversion.	Catabolic metabolites (Lactic acid, ethanol, …) Anabolic metabolites (aminoacids, nucleotides … and secondary metabolites (antibiotics, virulent factors). Genetically modified microorganisms	High specific activity of cell. High cell concentration (Fed batch and cell recycling) Extractive fermentation if Product inhibitor. Divided feed and fed batch if Substrate is inhibitor
Functional products (FP)	Standard raw matter and substrates Safety, stability and reproducibility of biochemical profiles.	GRAS status of microorganisms Genetically modified microorganisms	Validation of methods. Standardization of operating conditions. High productivity with fed-batch
Biodegradation strategies (BD)	Favour the use and recycle before biodegradation Maximize the removal of pollution with lower cost Find the worst yield of cell production	Enlarge the trophic capacity of consortia Maximize the affinity of cells to pollutants Control the stability of populations Find the optimal utilization of maintenance concept.	Both hydrodynamic rationality and oxygen transfer rate. SBR, Cell recycling flocculation, MBR. Steps anaerobic/ aerobic Bioaugmentation Chemostat implantation.

HACCP : Hazard Analysis Critical Control Point, GMP : Good Manufactoring Process, GHP: Good Hygiene Pratices, GRAS: Generally Recognized As Safe, EPS: Exopolysaccharides, SBR: Sequential Batch Reactor, MBR: Membrane biorecator.

Table 3: Steps involved in the industrial microbial processes

StepsIndustrial microbial processes			
Fermented foodCell and metabolites production waste treatment			
Pretreatment (Step 1)	Raw food treatment	Medium treatment	Raw waste treatment
Microbial Conversion (Step 2)	Fermentation (**batch**) mixed strains	Aerobic or anaerobic (**fed-batch**) pure strain	Aerobic or anaerobic (**SBR, continuous..**) consortium
Product Recovery (Step 3)	Fermented food	Cells or molecule	Treated waste
Goal	**Reproducibility, Nutritional**	**Product titer and Productivity**	**Pollution and safety removal**

4.3 Waste biodegradation

The aerobic and anaerobic oxidations of organic and mineral pollutant are carried out by different microorganisms using variable terminal acceptor electron (Table 1). The organic pollution especially caused by biodegradable and recalcitrant molecules are mineralized by the heterotrophic microorganisms. The biodegradation of polluting molecules in the natural environment is carried out by mixed microbial strains. The source and the dilution factor of solid, liquid and atmospheric pollution has a greatest impact on the wastewater disposal and treatment strategy (Fig. 3c). Moreover, certain components have influence on the plant design and management especially the suspended and floating solids, COD/BOD ratio, and nutrients salts such as ammonia, phosphate and sulfate which are considered vital for microorganisms growth [38]. The composition of a waste stream contributes to natural selection in wastewater bioreactor, a unique ecosystem evolves over time to degrade the organic compounds in each particular stream [27]. The solid wastes are fermented by batch techniques. Liquid and gas wastes are generally treated by continuous bioreactors with high cell density. The high levels of active biocatalysts in a biomass recycle reactors (BRR) can lead to significantly increased rates of wastes processing [9]. The exploitation of the biowaste as a renewable resource for bioproduct development could be a major challenge for biotechnology [39].

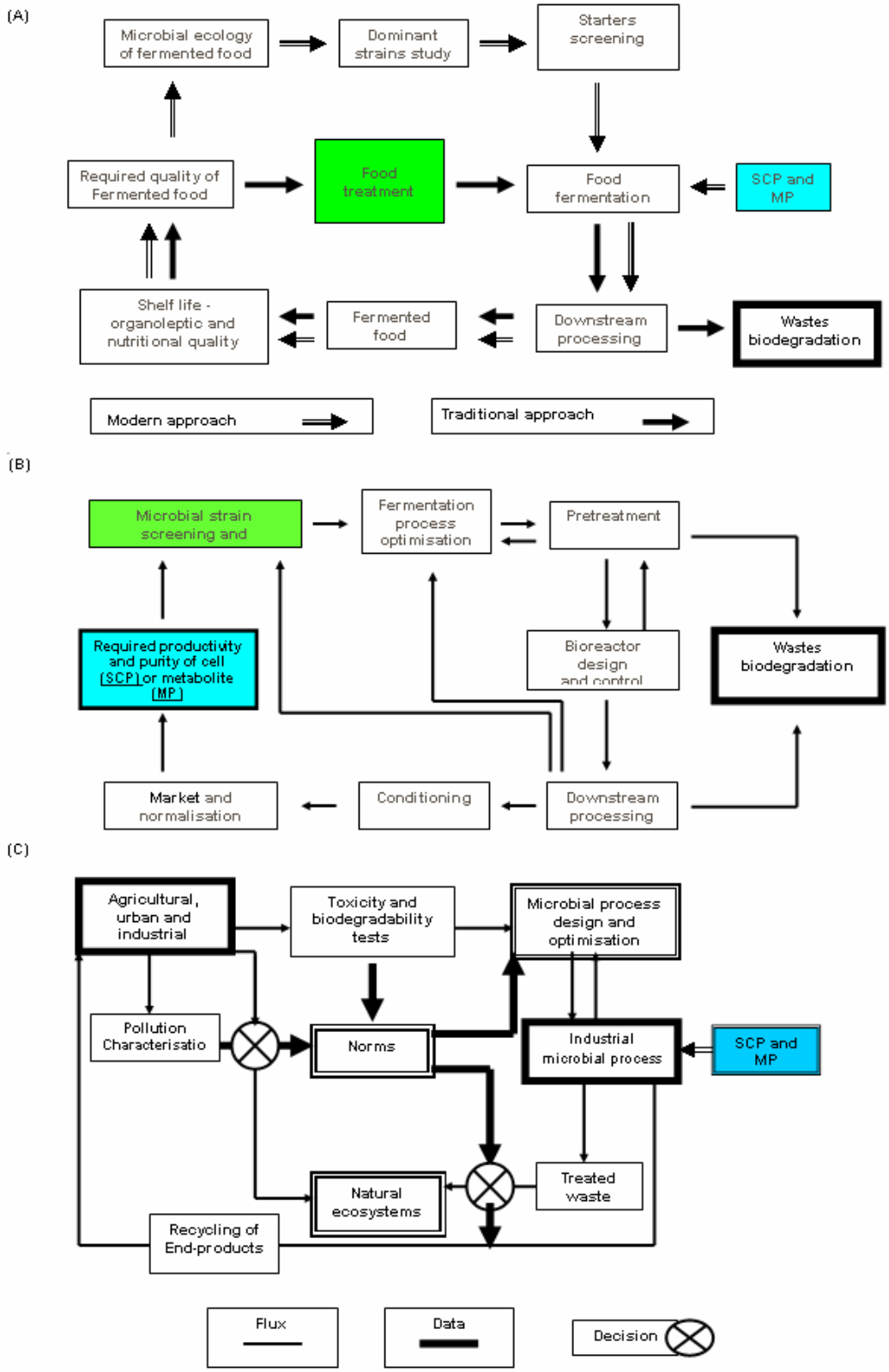

Fig. 3. The interactions between the different departments involved in the development of the industrial microbial process. (a) fermented food (b) cell and metabolites production (c) and wastes bioprocessing.

5. Conclusion and future prospects

There is no doubt that the study of microbial proteome according to environmental condition changes improve the industrial use of microorganisms. The selection of new industrial microorganisms will almost certainly require multiple approaches depending of the state of progress of genome and proteome characterization and their use will require efficient production methods. The membrane technology should contribute in the improvement of the bioreactor performance by increasing the cell density and their specific activities.

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