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Nutrient Optimization for the Production of Microbial Flocs in Suspended Growth Bioreactors

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ABSTRACT

Four successive experiments were carried out to determine the best combination of nutrients that could be added to sea water to enhance microbes to produce biofloc. In the first experiment, four levels of Nitrogen/ Phosphorus (N/P) ratio were tried with two sets of treatments for the four levels; with and without chicken manure. The level 4:1 from the second treatment was significantly (*P* <0.001) higher in dry weight (DW) and crude protein (CP) than the others. The second experiment aimed to using five levels of carbon/ nitrogen (C/N) ratios. The level (16:1) differed significantly, it was the highest in DW but its CP was slightly lower (*P* <0.001). This level was used in the third experiment with different carbon sources. The obtained DW from the rice meal was slightly higher, in contrast, the CP of the corn meal was the highest (*P* <0.001). In the fourth experiment a larger scale production of biofloc units using the best carbon source were used in different water sources. DW and CP of the biofloc for both trials were weakly significant (*P* <0.526). The nutritional composition of microbial flocs produced showed that CP was the highest component (39%). The study suggested that microbial protein could replace fishmeal or other protein sources in fish and shrimp feeding due to its good nutritional value and appropriate amino-acid profile which make it suitable for feeding omnivorous cultured species.

Keywords: Biofloc, microbial protein; bioreactor.

INTRODUCTION

In aquaculture practices, large quantities of wastes are produced; containing solids (e.g. feces and uneaten feed) and nutrients (e.g. nitrogen and phosphorus). This can be detrimental to the environment by causing eutrophication (Wetzel, 2001) or/ and by being toxic to aquatic fauna (Kuhn *et al*., 2010), if managed improperly. Many practices were applied to improve water quality of farms. One of these good practices is the biofloc technology (BFT) which could offer a solution to the problems resulted (De Schryver *et al*., 2008). If carbon and nitrogen are well balanced in the water, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass (Schneider *et al*., 2005). By adding carbohydrates, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (Avnimelech, 1999).

Biofloc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed (Hargreaves, 2006). This promoted nitrogen uptake by microbial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). Immobilization of ammonium by heterotrophic bacteria occurs much more rapidly because the growth rate and microbial biomass productivity per unit substrate of heterotrophic organisms are a factor 10 higher than that caused by the nitrifying bacteria (Crab *et al*., 2012).

Biofloc is composed of a variety of microorganisms, uneaten feed, feces, and detritus, and the particles are kept in suspension with water propulsion and aeration. Biofloc offers numerous ecological advantages for microbes, including protection from predators, direct access to nutrients, and necessary substrate area (De Schryver *et al*., 2008). It is thought to provide a packaging of microbial proteins and nutrients that is directly accessible to culture animals (Avnimelech, 2009; Burford *et al*., 2004). Biofloc technology (BFT) can be considered a culture technique in which water quality is maintained and in situ animal feed is simultaneously produced in the form of biofloc particles (Crab *et al*., 2007).

Biofloc technology makes it possible to minimize water exchange and usage in aquaculture systems through maintaining adequate water quality within the culture unit, while producing low cost protein-rich bioflocs, which can serve as a feed for aquatic organisms (Crab *et al*., 2012). Compared to conventional water treatment technologies used in aquaculture, biofloc technology provides a more economical alternative (decrease of water treatment expenses in the order of 30%), and additionally, a potential gain on feed expenses (the efficiency of protein utilization is twice as high in biofloc technology systems when compared to conventional ponds). Therefore, biofloc technology is a low-cost sustainable constituent to future aquaculture development (Avnimelech, 2009). It could also be used in the specific case of maintaining appropriate water temperature, good water quality and high fish/shrimp survival in low/no water exchange, greenhouse ponds to overcome periods of lower temperature during winter (Crab *et al*., 2012). In addition, Crab *et al.* (2010b) have recently shown that biofloc technology constitutes a possible alternative measure to fight pathogens in aquaculture.

Due to the great advantages of the biofloc technology, it has been applied in fin fish farming (Azim *et al.*, 2008; Avnimelech and Kochba, 2009; Schrader *et al*., 2011 and Widanarni *et al.*, 2012), wastewater treatment (Guo *et al*., 2010; Khezry, 2012 and Luo *et al.*, 2013) and shrimp farming (Megahed, 2010; Nunes and Castro, 2010; Ray *et al*., 2011; Bauer *et al*., 2012; Emerenciano *et al*., 2012; Moss *et al*., 2012; Baloi *et al*., 2013 and Emerenciano *et al*., 2013). The power requirement for mixing and aeration far exceeds that for conventional ponds and most recirculating systems (Hargreaves, 2013). Since, intensive aeration rates could not be applied to earthen ponds without significant erosion; most biofloc systems are lined (Hargreaves, 2013).

This study evaluates the effect of nutrient addition on the productivity and nutritional value of the biofloc produced in indoor bioreactors. It also aims to determine the best combination of nutrients that could be added to sea water to enhance microbes to produce biofloc which can be used in further studies for fish or shrimp feeding.

MATERIALS AND METHODS

Effect of addition of nutrients on the productivity and nutritional value of the biofloc

1. Experimental setup

Four successive experiments were carried out. Nutrients used were: urea (as a source of nitrogen with a 45% N), super phosphate (as a source of phosphorus with 20% P), chicken manure (as an organic fertilizer) and corn starch, corn meal, rice meal and rice bran (as a source of carbon). These nutrients were used with varying ratios in order to obtain the best productivity and nutritional value of microbial protein from the biofloc.

In the first experiment, four levels of Nitrogen/ Phosphorus (N/P) ratio were used. The concentration of nitrogen added to the sea water was changed in each level keeping the amounts of the other nutrients constant. The best N/P level that gave the best productivity and nutritional value was then selected and used in the following experiment. The second experiment aimed at using five levels of carbon/ nitrogen (C/N) ratios, only the carbon ratio was variable while the quantities of the other nutrients remained unchanged. The best C/N ratio was used in the third experiment with different carbon sources. In the fourth experiment, a large scale production of biofloc using the best carbon source in different water sources were carried out. Experiments took place in the Mariculture laboratory, at the department of Marine science, Suez Canal University, Ismailia.

Seawater with 30‰ salinity collected from the Beach Club, Ismailia was used for all experiments. In the laboratory, two steel chains were fixed and hung from the ceiling. To which, a long ruler-shaped metal piece (1.5m long) with fifteen slots was attached. Two fluorescent lamps were hung between the two chains. Polyethylene bags filled with ten liters of seawater were hung to the metal piece by means of hooks. These bags rested on a table to compensate the weight. The water inside the bags was agitated using a silicone tube connected to an air blower. The top of the bags were loosely tied up with straps.

2. Production of biofloc

Productivity of biofloc was assessed in terms of biomass. At the end of each experiment, bags were opened by cutting their upper parts to let the supernatant water flow out leaving the rest of water which contains the biofloc at the bottom. This water was poured into plastic bottles and placed in the refrigerator for 24hrs. Next, excess seawater was decanted away and the concentrated biofloc was poured in Petri dishes. They were then dried overnight in a laboratory oven at 70°C. The dried biofloc was weighed using an electrical balance then transferred into small plastic bags and kept in the freezer at (-4°C) for further analysis. The same procedure was used in experiment 4 except that the concentrated biofloc was dried in a laboratory oven for 48 hrs. After each experiment, the biofloc was analyzed for the proximate composition to determine the best level of nutrients in terms of productivity and nutritional value that could be used in the next experiment.

3. Experiment 1: Determination of the best ratio of Nitrogen/ Phosphorus (N/P)

Four ratios of N/P were tried: 1:1, 2:1, 4:1 and 8:1. Two sets of treatments for the four levels were made (using three replicates for each level). Twelve polyethylene bags were used for each treatment; the first group of bags had no organic fertilizer, while 0.05g chicken manure was added to each bag of the second. Added nutrients were the same in both sets: 0.025g of super phosphate and 0.1g of corn starch. The amounts of added urea were calculated according to the used percentage of phosphorus so that it became relative to each of the four ratios, they were: 0.02g, 0.04g, 0.08g and 0.15g to the ratios: 1:1, 2:1, 4:1 and 8:1 respectively. This experiment was carried out within the period from 8/7/2012 to 21/7/2012.

4. *Experiment 2: Determination of the best ratio of Carbon/ Nitrogen (C/N)*

Five ratios of C/N were tried: 1:1, 2:1, 4:1, 8:1 and 16:1 with three replicates for each. Fertilizers added were those used for the 4:1level in the second treatment of the N/P experiment. 0.08g of urea, 0.025g of super phosphate and 0.05g of chicken manure were used. The amounts of corn starch were also calculated according to the used percentage of urea so that it became relative to each of the five ratios, they were: 0.04g, 0.15g, 0.3g, 0.6g and 1.2g to the ratios: 1:1, 2:1, 4:1, 8:1 and 16:1 respectively. This experiment was carried out within the period from 27/9/2012 to 11/10/2012.

5. *Experiment 3: Determination of the best carbon source*

Three different sources of carbon were used, namely; corn meal, rice bran and rice meal with three replicates for each. A fixed weight (1.2g) was added to each bag. Fertilizers added were those used for the 16:1level of the C/N experiment. 0.08g of urea, 0.025g of super phosphate and 0.05g of chicken manure were used. This experiment was carried out within the period from 10/11/2012 to 23/11/2012.

6. *Experiment 4: Production of biofloc in larger units with different water sources*

Six 100L plastic tanks were used, three replicates for each water source (fresh and sea water). Freshwater sample was collected from Al-Raswa Farm. Water temperature was adjusted at 30°C ± 1. Fertilizers added were those used for the 16:1level of the 3rd experiment but multiplied by ten considering the volume factor (100L instead of 10L). 0.8g of urea, 0.25g of super phosphate, 0.5 g of chicken manure and 12 g of rice meal were used. Water agitation was provided by an air blower. This experiment was carried out within the period from 21/1to 4/2/2013.

7. *Determination of the nutritional composition of biofloc*

Biofloc was analyzed for the proximate composition using the Association of Analytical Chemist Methods (**AOAC, 2000**). The analysis was carried out at the Regional Center for Food and Agriculture (RCFF), Agriculture Research Center (ARC), Giza, Egypt. Moisture content was determined by drying the biofloc in an oven at 85°C until a constant weight was reached. Crude protein was determined according to Kjeldahl Method **(AOAC, 1990)**. Total fat was determined according to **Floch *et al.*, (1957)**. The carbohydrate content was estimated according to **Merrill and Watt, (1973)**: carbohydrate=100− (ash+ crude protein+ moisture+ total fat). Amino acid profile was determined according to **AOAC, (1990).** Mean values in the 4th experiment were calculated for three freshwater and three seawater replicates.

8. Identification and imaging of microorganisms groups in the biofloc

A water sample from the sea water tank was taken and diluted with pre-filtered water from the same tank. The sample was examined underneath a light microscope and different microorganisms were identified into groups. Another sample was taken, stained with Acridine orange according to Acridine orange direct count (AODC) method (**AOAC, 2000**) then examined and photographed at a magnification power 40 underneath a fluorescent microscope for the detection of bacteria. Images of bacterial floc were taken using a digital camera connected to a desktop computer and attached to the microscope. Imaging took place at the Biotechnology Research Center, Suez Canal University, Ismailia.

9. Statistical analysis

Data was transformed into $\log^{10}$ for computations. One way and two ways analyses of variance (ANOVA) were performed using **SYSTAT (V.10.2.05, 2002)** to determine the effect of nutrients addition on the productivity and quality of biofloc. The 5% significance ratio was applied to all experiments. Differences among means of the obtained data were assessed by the Tukey post hoc tests (**Zar, 1996**) (alpha = 0.05).

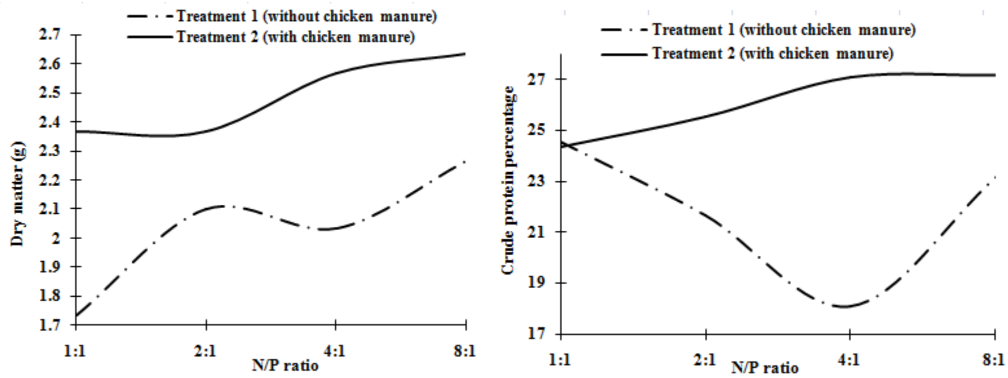
10. Cost analysis

Calculations of the production cost of biofloc meal were carried out based on the level of nutrients that yielded the highest dry weight. The cost was calculated for a large scale system of a 1,000 square meters pond with one meter depth. Costs included those for pond preparation and equipments as well as operational ones such as price of nutrients and running the system.

RESULTS

1. The best Nitrogen/ Phosphorus (N/P) ratio

The produced amount of biofloc (3g) was sufficient enough to estimate the percentage of crude protein only. Dry weight (DW) and crude protein percentage (CP) of the biofloc produced from the four levels of the second treatment were higher than those of the first treatment (Fig. 1 and 2). The highest DW (2.6g) and CP (27%) was produced from the second treatment; however, N\P levels of 4:1 and 8:1 showed approximately the same DW and CP. Dry weight and crude protein percentage of the biofloc produced were significantly different (P <0.001) from the two treatments.



(Figs.1 and 2): ***Dry weight and crude protein percentage of the biofloc obtained at different levels of N/ P ratios.***

1. The best Carbon/ Nitrogen (C/N) ratio

The four C/N levels (1:1, 2:1, 4:1and 8:1) had minor differences in DW content, in contrast, they differed greatly in CP percentages (Figs. 3 and 4). The level (2:1) was higher in DW (3.8g) as well as CP (35.3%) than the others. However, the fifth trial (16:1) showed the highest DW value (9g) with a relatively lower percentage of CP (25.3%). Differences in the DW and CP values between the five levels were significantly different (*P* <0.001).

2. The best carbon source

The DW obtained from the rice meal was higher than corn meal and rice bran (Figs. 5 and 6). In contrast, the CP of the corn meal was higher than rice bran and the rice meal. Variations in the DW and CP values between the three carbon sources were significantly different (*P* <0.001).

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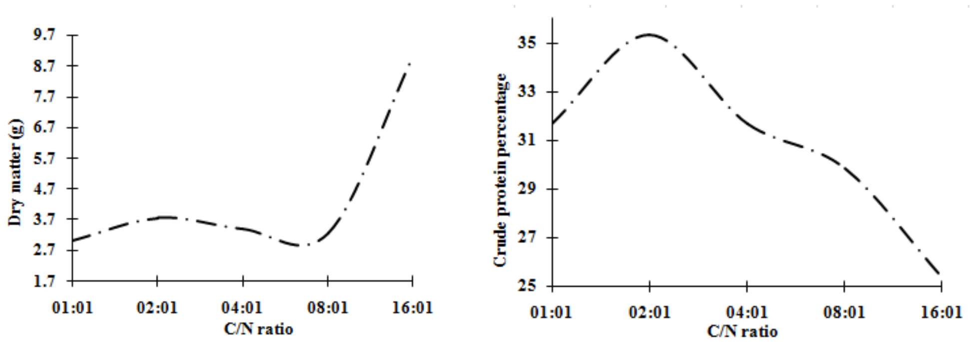
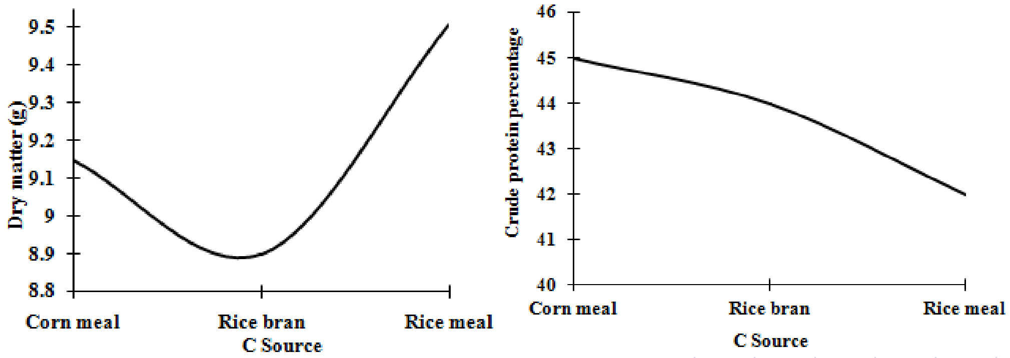


(Figs. 3 and 4): ***Dry weight and crude protein percentage of the biofloc obtained at different levels of C/N ratios.***



(Figs. 5 and 6): ***Dry weight and crude protein percentage of the biofloc obtained from different carbon sources.***

-Amino acids profile

Corn meal exhibited higher values of amino acids than those of rice meal and rice bran (Table 1). Some amino acids (e.g. Cystine and DL-Methionine) were absent (or found in undetected concentrations) in the biofloc produced from corn meal and rice bran. Differences in amino acid profile values between the three carbon sources were significantly significant (*P* <0.001).

4. Production of biofloc in larger units with different water sources

DW and CP of the biofloc produced from the seawater were slightly higher than those obtained from fresh water (Table 2). Differences of the DW and CP values of the biofloc from both trials were not statistically significant (*P* <0.526, *P* <0.184).

- ***Nutritional composition of bioflocs***

Crude protein was the highest component in the biofloc (39%) (Table 3), followed by carbohydrates (30%) then ash (18%). Crude fiber and fat were the lowest components obtained.

Table 1: Amino acids profile (expressed as mg/ 100 mg dry weight) of the biofloc obtained from the different carbon sources.

	Essential amino acid									Nonessential amino acid							
Amino acid	**Leucine**	**Lysine**	**Argnine**	**Valine**	**Histidine**	**Therionine**	**Isoleucine**	**Phenylalanine**	**DL-Methionine**	**Glutamic**	**Aspartic**	**Proline**	**Alanine**	**Glycine**	**Tyrosine**	**Cystine**	**Serine**
Corn meal	2.5	1.4	1.3	2	0.6	1.4	1.5	1.1	-	4	2.6	1.7	1.9	1.5	0.9	-	1.4
Rice bran	1.3	0.8	0.9	1.4	0.3	0.8	0.7	0.6	-	1.9	1.5	0.8	1	0.9	0.4	-	0.8
Rice meal	1.7	1.6	1.2	1.2	0.3	1	0.9	0.8	0.23	3.6	2.4	1.1	1.4	1.3	0.2	0.47	1.3

Table 2: ***Dry weight values of biofloc and their crude protein percentages produced from fresh and sea water.***

Source of water	Dry Weight of biofloc (g) (mean±SD) (*10)	Crude Protein percentage of biofloc (mean± SD)
Seawater	9.4±0.21	41.7±0.72
Freshwater	9.3±0.38	39.2±2.6

Legend: SD: Standard deviation.

Table 3: ***Nutritional composition of biofloc, mean values and standard errors.***

Nutritional composition of biofloc (n= 6)	Crude protein	Carbohydrates	Crude fat	Crude fiber	Total ash	Total
mean± SD (g/100g)	**39± 1**	**30± 1.7**	**1.1± 0.1**	**13± 0.5**	**18± 2.2**	**Approximately 100**

Legend: SD: Standard deviation; n: number of replicates.

5. Identification and imaging of microorganisms groups in the biofloc

Six groups of organisms were identified: chlorophytes, diatoms, dinoflagellates, nematodes, rotifers, and cyanobacteria (Plate 1).

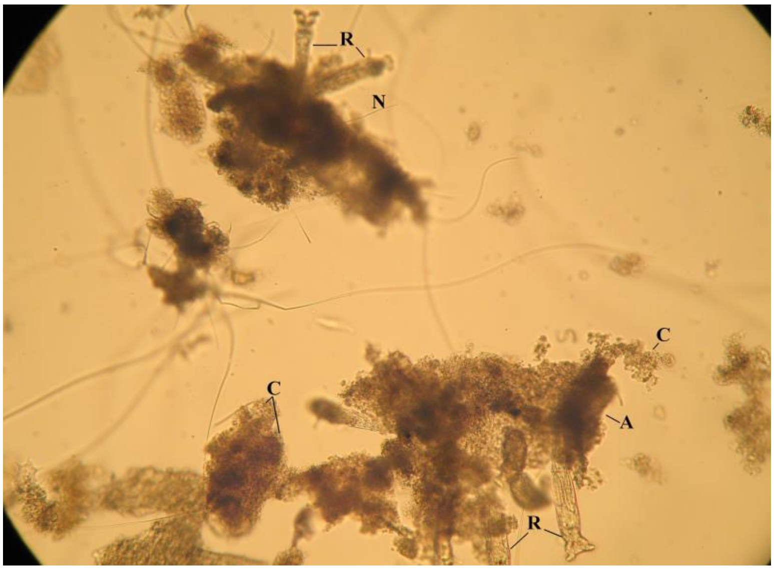


Plate 1: Composition of biofloc, A: biofloc particle, C: chlorophytes, N: nematode and R: rotifers.

Plate 1 shows the structure of a biofloc particle and the different floc associated organisms. (A) a biofloc particle, is a mixture of micro-organisms (floc-formers and filamentous bacteria), particles, colloids and dead cells. (B) high abundance of chlorophytes, also called green algae, nematodes (N) and rotifers (R). Nematods were observed feeding on the flocs in fresh samples examined under the microscope. Fluorescent images clearly showed the clusters of stained bacterial cells which in the biofloc (Plate 2). Flocs' particles varied in size ranging from 50 to 200 μm.

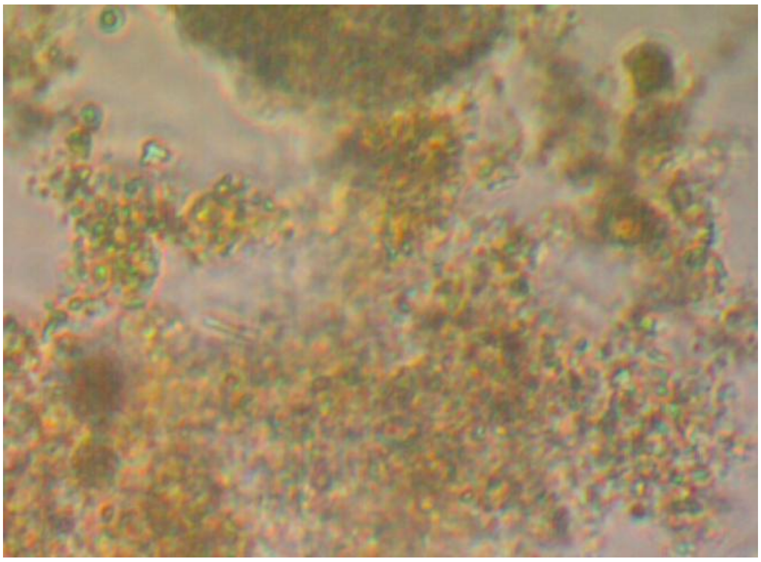


Plate 2: ***A fluorescent image of a 14-days-old bacterial floc stained with Acridine orange.***

6. Cost analysis

The level of nutrients 16:1 of the 3rd experiment yielded the highest dry weight of biofloc (9.51g) (Fig. 5) and was therefore used to calculate the costs. Establishing costs of this system was based on two major categories; fixed and operational costs.

The sum was then multiplied by 1000/9 in order to find the total cost of one kilogram of biofloc meal in Egyptian pounds as follows:

- Cost of added nutrients = 0.003 LE.
- Cost of one kilogram of biofloc meal= 1000/9 X 0.003 = 0.333= 33 pt.
- Cost of biofloc meal per ton= 0.333 X 1000 = 333 LE.

The calculation of the total expenses for the production of one ton of biofloc meal including the operating costs during the 15 days experimental period was as follows:

Cost of pond liners + Aeration cost + Power cost + Labor cost

Cost category	Description	Amount (LE)
Fixed costs	1. Lining the pond with a half millimeter thickness polyethylene sheet	11000 LE
	2. One horse capacity aerator	3000 LE
Operational costs	1. Energy of running the aerator per month	100 LE
	2. The price of nutrients per kilo gram as follow;	
	1 Kg urea	3 LE
	1 Kg super phosphate	2 LE
	1 Kg chicken manure	0.5 LE
	1 Kg broken rice	2 LE
	3. Labor cost per month	200 LE

The cost of added nutrients was calculated by multiplying the amount of each type by its price.

Nutrient	Cost
Urea	0.08 * 0,003 = 0.00024 LE
Super phosphate	0.0025 * 0,002 = 0.000005 LE
Chicken manure	0.05 * 0.0005 = 0.000025 LE
Broken rice	1.2 * 0.002 = 0.0024 LE
Total	0.003 LE

These are added to the cost of nutrients used: 11000+ 3000+ 100+ 200+ 333 = 14633 LE. For the regular farming period in Egypt (six months), expenses reach up to 21596 LE according to the following equation:

Cost of pond liners + Aeration cost + [(Nutrients cost + Labor cost + Power cost) X 2 tons X 6 months] =

11000 +3000 + [(333+ 200+ 100) X 2 X 6] = 21596 LE

To calculate the cost of one ton, the total expenses was divided by 12 as follow; 21596/ 12 = 1800 LE of biofloc meal per ton, which is equivalent to 257$ U.S.A according to the exchange rate in April 2014.

Assuming that this system is usable for 3 years, the cost can be calculated as follows: 11000 +3000 + [(333+ 200+ 100) X 3 X 12] = 36788 LE
The cost per ton = 36788/36 tons = 1021 LE, which is equivalent to 143$ U.S.A at the exchange rate in April 2014.

DISCUSSION

Biofloc technology (BFT) has been used as a promising alternative grow-out systems and a method for obtaining protein for diets that originated from the diverse mico-organisms (**Emerenciano *et al*., 2013**). The present study aimed to produce an inexpensive biofloc meal that could be used in aquaculture systems and to evaluate its nutritional characteristics under fully controlled conditions.

1. The best Nitrogen/ Phosphorus (N/P) ratio

The obtained results revealed that the highest dry weight content and crude protein percentages were produced from the levels 4:1 and 8:1 in the second treatment (with organic manure). This result is in accordance with that reported by **Hepher and Pruginin, (1981)** who applied different N/P levels in a fish pond in Israel and reported that the healthiest N/ P ratio was 4:1. They concluded that levels of P over 0.4 mg/l and over 1.5 mg/l in the water were not useful in increasing the productivity of the pond. This high value of crude protein recorded in the present study can be referred to the addition of organic manure. Organic manure, which includes detritus, has been reported to be a rich source of nutrients (**Wade and Stirling, 1999b**). The values obtained from the levels 4:1 and 8:1 were approximately the same. This implies that organic manure should be used in limited quantities since its excessive addition is useless. **Boyd and Massaut, (1999**) reported that the decomposition of manure by bacteria requires oxygen, and the amount of manure that can safely be added to a pond depends on the biochemical oxygen demand of the manure. They explained that adding manures to a pond increases the potential for low oxygen concentrations in pond water and pond effluents. When applying organic manure to ponds, they may serve as direct sources of food for invertebrates and fishes, or they may decompose slowly to release inorganic nutrients that stimulate phytoplankton growth. Excessive growth of phytoplankton or eutrophication will eventually change the water quality of the pond, thus leading fish farmers to increase the aeration which is quite costly.

2. The best Carbon/ Nitrogen (C/N) ratio

The level 16:1yeilded the highest value of dry weight (9g) and a considerably high percentage of crude protein (25.3%). It is therefore suggested that this level could be used as a replacement of fishmeal in the diet of farmed organisms particularly shrimps since they need a high protein diet. **Fontenot *et al*., (2007)** used four C: N ratios (5:1, 10:1, 20:1 and 30:1) in treating shrimp aquaculture wastewater in South Carolina by adding molasses or ammonium salt. The wastewater was successfully treated using a sequencing batch reactor (SBR). They reported that the C: N ratio of 10:1 gave the best results in terms of maximum inorganic nitrogen removal from wastewater. However, they did not quantify the bacterial biomass in this optimum C: N ratio since the objective of the experiment was to treat wastewater rather than producing microbial protein. On the other hand, the level 2:1 in the present study gave the highest percentage of crude protein (35.3%) and the lowest content of dry weight (3.8g).

Crude protein percentages of the biofloc collected from the different trials were within the range of those which have been previously reported (**Azim and Little, 2008;**

De Schryver and Verstraete, 2009; Crab *et al*., 2010; Ekasari *et al*., 2010). **Widanarni *et al*., (2012)** reported that protein requirement for grow out culture of red tilapia in outdoor concrete tanks, Indonesia, varied from 20 to 42%. Consequently, the present study proposes the introduction of the BFT in the farming trials of red tilapia in Egypt and encourages the use of the C/N level 16:1 as it could fulfill the dietary requirement of this fish.

3. *The best carbon source*

The results showed that the crude protein percentage of the biofloc produced from the first trial (with corn meal as a carbon source) was the highest (45%), followed by rice bran (44%) and rice meal (42%), respectively. In contrast, the dry weight of biofloc produced from rice meal (9.51g) was higher than that of corn meal (9.15g) and rice bran (8.9g), respectively. These findings were similar to the CP content obtained by **Ekasari *et al.*, (2010)** who used two carbon sources; sugar and glycerol, as the first variable, and two different levels of salinity, 0 and 30 ppt, as the second variable to produce biofloc in reactors in Belgium. The reactors in which the BFT was applied consisted of rectangular plastic containers. They reported that crude protein of the biofloc from both trials were in a range of 18-42% and concluded that there was no effect of both organic carbon sources on the crude protein of the biofloc.

The amino acid profile of the biofloc produced showed that corn meal had higher values of amino acids than rice meal and rice bran, respectively. However, some of the essential amino acids (such as DL-Methionine) and nonessential amino acids (such as Cystine) were missing from that produced from corn meal and rice bran. Our results are consistent to those given by **Logan *et al*., (2010)** who performed two feeding trials on the Pacific white shrimp *Litopenaeus vannamei* at Texas Agrilife Mariculture Lab in USA. One feed was formulated from biofloc as a protein source with no fishmeal and another with fishmeal. They recorded excellent growth and health in animals that consumed microbial protein in their diet and concluded that the protein content and amino acid composition of the biofloc was very similar to those of fishmeal.

The present study suggests that rice meal could be used as a carbon source in farming organisms in Egypt. Firstly because it's a local produce, secondly, it's cheaper than corn since fish farmers use broken rice, and thirdly, because most of the corn species imported by Egypt are genetically engineered ones which would eventually impact farmed species. **El-Shamei *et al*., (2012)** studied the histopathological changes in some organs of male rats fed on genetically modified corn (GM) and reported that liver, kidneys, testes, spleen were badly affected. They suggested further investigations of the possible effects of GM food consumption by farmed species which might help in the market surveillance.

4. *Production of biofloc in larger units with different water sources*

No significant differences were found between the two sources of water. The mean crude protein percentage of the biofloc in fresh and saline water was (33%). This was in correspondence with other studies carried out in saline water (**Tacon *et al*., 2002; Ju *et***

***al*., 2008; Ekasari *et al.*, 2010**), as well as, in freshwater **(Vanstechelman, 2008)**.

The mean carbohydrate content from both trials was 30% while crude fiber was 13%. According to **Emerenciano *et al*., (2013)**, the protein, carbohydrates, fiber and ash content in biofloc particles could vary substantially (12 to 49, 18 to 36, 0.8 to 16.2 and 13 to 46%, respectively). The value of crude fat of the biofloc produced in the present study was within the range of those which have been previously reported (**Kuhn *et al*., 2009; 2010).**

The mean ash content of the biofloc was 22%, which agreed with other studies, (**Tacon *et al*., 2002; Azim *et al*., 2007; Ju *et al*., 2008**). **Tacon *et al*., (2002)** suggested that the high ash content in the biofloc was probably related to the presence of acid insoluble oxides and mixed silicates.

5. Identification and imaging of microorganisms groups in the biofloc

Six groups of organisms were identified: chlorophytes, diatoms, dinoflagellates, nematodes, rotifers, and cyanobacteria. This result is in accordance with **Ray *et al*., (2010),** who studied the characterization of microbial communities in minimal-exchange, intensive aquaculture systems and reported that the abundance of chlorophytes was higher than diatoms and heterotrophic dinoflagellates. They attributed the reason to the competition for resources existed between chlorophytes and diatoms. On the other hand, nematodes and rotifers were observed grazing on the floc particles. Such observation reflects the vitality of the biofloc community in which the interactions between organisms take place. Cyanobacteria were detected by means of fluorescent microscope. This finding has been documented by other studies **(Azim and Little, 2008; Azim *et al*., 2008; Ray *et al*., 2010). Logan *et al*., (2010)** reported that bacterial generation times are much shorter than those of yeasts or algae, often on the order of 20 to 30 minutes rather than the hours or even days required by the other organisms.

6. Cost analysis

According to the potential estimates of the costs of producing a metric ton of dry weight from the biofloc meal is approximately 143$. The global soy meal market varied approximately from $470 to $615/ metric ton in April 2014. During the same period, fishmeal varied approximately from $1500 to $2000, suggesting feasibility on replacement of either soybean and/or fish meal by biofloc meal.

Overall, the study showed that the nutritional composition of biofloc can be sufficient to omnivorous organisms in terms of satisfying their needs of protein and essential amino acids, therefore analyzing the factors affecting as well as other nutritional parameters such as fatty acid profile and vitamins which are important in fish or shrimp nutrition are of interest.

Biofloc technology will enable aquaculture grow towards an environmental friendly approach. Consumption of microorganisms in BFT reduces costs in feed. Also, microbial community is able to rapidly utilize dissolved nitrogen leached from shrimp faeces and uneaten food and convert it into microbial protein. These qualities make minimal-exchange BFT system an alternative to extensive aquaculture. Biofloc might partially replace protein content in diets or decrease its dependence of fishmeal. This study encourages fish farmers to invest in this novel technique as it is financially profitable.

REFERENCES

AOAC (Association of Analytical Chemists) (1990). Official Methods of Analysis, 15th Edition, Washington, D.C.; U.S.A.

AOAC (Association of Analytical Chemists) (2000). Official Methods of Analysis, 17th Edition, Washington, D.C.; U.S.A.

Avnimelech, Y. (2009). Biofloc Technology — A Practical Guide Book. *The World Aquaculture Society*, Baton Rouge, Louisiana, United States. 182 p.

Avnimelech, Y., (1999). Carbon and nitrogen ratio as a control element in Aquaculture systems. *Aquaculture*, 176; pp. 227–235.

Avnimelech, Y., and Kochba, M. (2009). Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using ^{15}N tracing. *Aquaculture,* 287; pp. 163–168.

Azim M. E., Little, D. C. and Bron, J. E. (2008). Microbial protein production in activated suspension tanks manipulating C: N ratio in feed and the implications for fish culture. *Bioresource Technology*, 99; pp. 3590–3599.

Azim, M. E. and Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 283; pp. 29-35.

Azim, M. E., Little, D. C. and Bron, J. E. (2007). Microbial protein production in activated suspension tanks manipulating C/N ratio in feed and implications for fish culture. *Bioresource Technology*, 99; pp. 3590-3599.

Baloi, M., Arantes, R., Schveitzer, R., Magnotti, C. and Vinatea, L. (2013). Performance of Pacific white shrimp *Litopenaeus vannamei* raised in biofloc systems with varying levels of light exposure. *Aquaculture Engineering*, 52; pp. 39– 44.

Bauer, W., Prentice-Hernandez, C., Tesser, M. B., Wasielesky Jr., W. and Poersch, L. H. S. (2012). Substitution of fishmeal with biofloc meal and soy protein concentrates in diets for the pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, 342–343; pp. 112–116.

Boyd, C. E. and Massaut, L. (1999). Risks associated with the use of chemicals in pond aquaculture. *Aquacultural Engineering*, 20; pp. 113–132.

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H. and Pearson, D. C. (2004). The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, 232; pp. 525–537.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P. and Verstraete, W., (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270; pp. 1–14.

Crab, R., Chielens, B., Wille, M., Bossier, P. and Verstraete, W. (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research*, 41; pp. 559-567.

Crab, R., Defoirdt T., Bossier P. and Verstraete W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, 356–357; pp. 351–356.

Crab, R., Lambert, A., Defoirdt, T., Bossier, P., Verstraete, W. (2010b). Bioflocs protect gnotobiotic brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. *Journal of Applied Microbiology*, 109; pp. 1643–1649.

De Schryver, P. and Verstraete, W. (2009). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresource Technology*, 100; pp. 1162 1167.

De Schryver, P., Crab R., Defoirdt T., Boon N. and Verstraete W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, 277; pp. 125–137.

DeVantier, L. M., De'ath, G., Done, T. J. and Turak, E., (1998). Ecological assessment of a complex natural system: a case study from the Great Barrier Reef. *Ecological Applications*, 8 (2); pp. 480–496.

Ekasari, J., Crab, R. and Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal of Biosciences*, 17; pp. 125-130.

El-Shamei, Z. S., Gab-Alla, A. A., Shatta, A. A., Moussa, A. and Rayan, A. M. (2012). Histopathological Changes in Some Organs of Male Rats Fed on genetically Modified Corn (Ajeeb YG). *Journal of American Science*, 8(10); pp. 684-696.

Emerenciano, M., Cuzon, G., Are´valo, M., Miquelajauregui, M. M. and Gaxiola, G. (2012). Effect of short-term fresh food supplementation on reproductive performance, biochemical composition, and fatty acid profile of *Litopenaeus vannamei* (Boone) reared under biofloc conditions. *Aquaculture International*, DOI 10.1007/s10499-012-9607-4.

Emerenciano, M., Cuzon, G., Goguenheim, J., Gaxiola, G. and Aquacop (2013). Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. *Aquaculture Research*, 44; pp. 75–85.

Emerenciano, M., Gaxiola, G. and Cuzon, G. (2013). Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry, Biomass Now - Cultivation and Utilization, Dr. Miodrag Darko Matovic (Ed.), ISBN: 978-953-51-1106-1, InTech, DOI: 10.5772/53902. Available from: http://www.intechopen.com/books/biomass-now-cultivation-and utilization/biofloc-technology-bft-a-review-for-aquaculture-application-and-animal-food-industry.

Folch, J., Lees, M. and Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Biological Chemistry*, 226; pp. 497-509.

Fontenot, Q., Bonvillain, C., Kilgen, M. and Boopathy, R. (2007). Effects of temperature, salinity, and carbon:nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. *Bioresource Technology*, 98; pp. 1700–1703.

Guo, F., Zhang, Sheng-Hua, Yu, X. and Wei, B. (2010). Variations of both bacterial community and extracellular polymers: The inducements of increase of cell hydrophobicity from biofloc to aerobic granule sludge. *Bioresource Technology*, 102; pp. 6421–6428.

Hargreaves, J. A. (2006). Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering*, 34; pp. 344–363.

Hargreaves, J. A. (2013). Biofloc production systems for aquaculture. *Southern Regional Aquaculture Center (SRAC Publication)*, No. 4503, USA.

Hepher, B. and Y. Pruginin. (1981). Commercial fish farming with special reference to fish culture in Israel. John Wiley & Sons, New York. 261 pp.

Ju, Z. Y., Forster, I., Conquest, L., Dominy, W., Kuo, W. C. and Horgen, F. D. (2008). Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquaculture Research*, 39; pp. 118-133.

Kastovska, K., Stibal, M., Sabacka, M., Cerna, B., Santruckova, H., Kastovska, K., Stibal, M., Sabacka, M., Cerna, B., Santruckova, H. and Elster, J., (2007). Microbial community structure and ecology of subglacial sediments in two polythermal Svalbard glaciers characterized by epifluorescence microscopy and PLFA. *Polar Biology*, 30; pp. 277–287.

Khezry, M. (2012). Mapping the distribution of the eps matrix within mixed bioflocs. M.Sc., University of Guelph, Ontario, Canada, 121 p.

Kuhn, D. D., Boardman, G. D., Lawrence, A. L., Marsh, L. and Flick, G. J. (2009). Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture*, 296; pp. 51–57.

Kuhn, D. D., Flick, Jr. G. J. and Lawrence, A. L. (2010). Biofloc: Novel sustainable ingredient for shrimp feed. *Global Aquaculture Advocate*, May/June 2010; pp. 71-72.

Kuhn, D. D., Lawrence, A. L., Boardman, G. D., Patnaik, S., Marsh, L. and Flick, G. J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei. Aquaculture*, 303; pp. 28–33.

Logan, A. J., Lawrence, A., Dominy, W. and Tacon, A. G. J. (2010). Single-cell proteins from food byproducts provide protein in aquafeed. *Global Advocate*, 13; pp. 56-57.

Luo, Guo-zhi, Avnimelech, Y., Pana, Yun-feng and Tan, Hong-xin (2013). Inorganic nitrogen dynamics in sequencing batch reactors using biofloc technology to treat aquaculture sludge. *Aquaculture Engineering*, 52; pp. 73– 79.

Megahed, M. E., (2010). The effect of microbial biofloc on water quality, survival and growth of the Green Tiger Shrimp (*Penaeus Semisulcatus*) fed with different crude protein levels. *Journal of The Arabian Aquaculture Society*, 5(2); pp. 1-24.

Merrill, A. L. and Watt, B. K. (1973). Energy value of foods: basis and derivation. United States Department of Agriculture (USDA), Handbook No. 74.

Moss, S. M., Moss, D. R. Arce, S. M., Lightner, D. V. and Lotz, J. M. (2012). The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. *Journal of Invertebrate Pathology*, 110; pp. 247–250.

Newall, P., Bate, N. and Metzeling, L., (2006). A comparison of diatom and macroinvertebrate classification of sites in the Kiewa River system, Australia. *Hydrobiologia*, 572; pp. 131–149.

Nunes, A. J. P. and Castro, L. F. (2010). Bioflocs spare protein in white shrimp diets. *Global Aquaculture Advocate*, May/June 2001; pp. 24-26.

Ray, A. J., Dillon, K. S. and Lotz, J. M. (2011). Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management. *Aquaculture Engineering*, 45; pp. 127– 136.

Ray, A. J., Seaborn, G., Leffler, J. W., Wilde, S. B., Lawson, A. and Browdy, C. L. (2010). Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture*, 310; pp. 130–138.

Schneider, O., Sereti, V., Eding, E. H. and Verreth, J. A. J., (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquaculture Engineering*, 32; pp. 379–401.

Schrader, K. K., Green, B. W. and Perschbacher, P. W. (2011). Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). *Aquaculture Engineering*, 45; pp. 118– 126.

SYSTAT (2002). SYSTAT for Windows. Version 10.2. Statistics. SYSTAT, Evanston, IL.

Tacon, A. G. J., Cody, J. J., Conquest, L. D., Divakaran, S., Forster, I. P. and Decamp, O. E. (2002). Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, 8; pp. 121-137.

Vanstechelman, H. (2008). Bio-vloktechnologie: Het verwijderen van nutrienten in de aquacultuur en de simultane productie van hoogwaardige nutritionele vlokken [Thesis]. Ghent, Belgium: Ghent University.

Wade, J. W. and Stirling, H. P. (1999b). Fertilization of earth ponds. I: Effects on physico-chemical characteristics and eutrophication of pond water and soil. *Journal of Aquatic Science*, 14; pp. 5-12.

Wetzel, R. G. (2001). Limnology: Lake and River Ecosystems, 3rd Edition. Academic Press, San Diego, California, US.

Widanarni, E. J. and Maryam, S. (2012). Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis sp.* cultured at different stocking densities. *Journal of Biosciences*, 19 (2); pp. 73-80.

Zar, J. H. (1996). Biostatistical Analysis, Second Ed. Prentice Hall, Englewood Cliffs, 662 p.

تحديد أفضلية المغذيات لإنتاج التجمعات الميكروبية في المفاعلات الحيوية

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تم اجراء اربع تجارب متعاقبة لتحديد أفضل المغذيات مضافة لمياه البحر لتعزيز كثافة الميكروبات لانتاج التجمعات الأحيائية (بيوفلوك). في التجربة الأولى تم عمل أربع مستويات من نسب النتروجين والفوسفور في مجموعتين الأولى في وجود سماد الدواجن والآخر بدونه، كانت مستوى نسبة النتروجين للفوسفور ذات قيمة ١:٤ وفي وجود سماد الدواجن ذو قيم أعلى من حيث المادة الجافة ونسبة البروتين. في التجربة الثانية تمت اضافة خمسة مستويات من نسب الكربون للنتروجين وكانت النتائج متباينة وكانت نسبة ١:١٦ أعلى من حيث كم البيوفلوك والأقل من حيث نسبة البروتين. وفي التجربة الثالثة تمت اضافة أنواع مختلفة من مصادر الكربون ولقد أعطت اضافة مسحوق الأرز أفضل نتائج من حيث الكم من البيوفلوك وكان البيوفلوك الناتج من اضافة مسحوق الذرة هو الأعلى من حيث نسبة البروتين. وكان الاختلاف غير مؤثر عند انتاج البيوفلوك في مياه متباينة من حيث الملوحة. وباجراء تحليل البيوفلوك كيميائيا وجدت الدراسة أن المحتوى البروتيني (٣٩%) هو الأكثر من بين المكونات المختلفة للبيوفلوك ولذلك فإن الدراسة تقترح استخدام مسحوق البيوفلوك كمصدر للبروتين وبديل لمسحوق السمك أو أي من مصادر البروتين في غذاء الأسماك والقشريات نظرا لجودة نوعية البروتين والتي تضم تركيبا جيدا في محتواه من الأحماض الأمينية والتي قد تجعله مناسبا لتغذية الأنواع المستزرعة وخاصة الغير مفترسة منها.

الكلمات المفتاحية : بيوفلوك، البروتين البكتيري، مفاعل حيوي.