EFFECT OF I_E SOLUTIONS ON ENZYMES AND MICROBIAL CELLS

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The effects of five I_E^{TM} water preparations (D_S, A_{CE}, S_S, I_M, C_S) of various concentrations on aerobic fungi and aerobic bacteria were investigated *in vivo,* by substituting I_E water for distilled water in culture media. It was established that the bioactivity of I_E waters depends on the type of I_E preparation, its concentration, and the type of microorganism used for fermentation. As much as 100% increase in maximum enzymatic activity and up to 30 hours decrease in fermentation time was observed in some microorganisms when culture media were prepared with certain types of I_E water. However, no effect or even inhibition of enzymatic activity by I_E was seen in some microorganism/I_E combinations. An optimal I_E preparation was identified for each microorganism for further scale-up experiments. The fungal colonies grown on agar plates in the presence and in the absence of I_E water demonstrated distinct morphological differences and thus, these findings indicate a significant bioactivity of I_E waters. The rate of methane production by a methanogenic consortium increased about 2 times over control when stimulated by D_S-50 I_E water. The effects of different types of I_E waters on cellulase activities (FPA, CMCase, β-glucanase, β-glucosidase), xylanase, α-amylase, glucoamylase and lipoxigenase activities were investigated *in vitro,* by substituting I_E water for 50-100% of distilled water in the enzymatic reaction mixtures. The most significant stimulating effect of certain I_E preparations (up to 80 %) was detected in the case of cellulases. Increase in enzymatic activity (15-70 %) was observed in the other enzymes following their stimulation with I_E waters.

1. Introduction

Microorganisms are generally divided in four major groups: bacteria, fungi (molds, yeasts, and mushrooms), algae, and protozoa. All of these organisms are single cell or clusters of cells of the same type. Microbial biotechnology, or industrial microbiology, deals with processes involving microorganisms, both naturally existing and genetically engineered. Pharmaceutical, Agricultural, Food, Chemical, and Environmental Industries widely use microbial products and processes (Figures 1, 2).

One of the important groups of industrial products are enzymes, globular catalytic proteins, highly specific in the reaction they catalyze. There is considerable evidence that a definite three-dimensional configuration is essential for the enzyme to work properly. The so called, lock-and-key model, postulates that the enzyme has a specific site, the "lock", which is a geometrical compliment of the substrate, "key", and that only substrates with the proper complementary shape can bind to the enzyme so that catalysis occurs.

There are thousands of enzymes presently known, however, the proteases, enzymes that hydrolyze peptide bonds in proteins, and the α-amylases, enzymes which hydrolyse starch, dominate the present commercial enzyme market. These enzymes are widely used in the laundry, dry cleaning, food, textile, paper, pharmaceutical, and other industrial applications to name a few.

Another group of enzymes with a large potential for industrial application are the cellulases, enzymes involved in hydrolysis of cellulose. The production of glucose from cellulose containing wood based materials and agricultural wastes is not yet profitable but is being intensively studied world-wide. Glucose, produced by hydrolysis of cellulose, can be fermented to produce ethanol and other chemical intermediates. Although the cellulase enzyme can be produced efficiently, the relatively low activity of the enzyme means that it must be used in large quantities to achieve adequate cellulose hydrolysis.

The activity of an enzyme defines its ability to catalyze a reaction. The higher the activity of an enzyme, the faster the reaction it catalyzes will go and/or smaller amount of the enzyme will be required to catalyze the same reaction.

Recently Lo[1] and Lo *et al*.[2] reported on distinct physical and chemical properties of stable I_E^{TM} water clusters (I_E water). Water is one of the most essential substances for living. Approximately 70% of a bacterial cell mass is water, while cells of higher organisms are as much as 90% water (Neidhardt *et al*.[3]). It is important, therefore, to study the biological activity of I_E water.

Major industries which make wide use of industrial microbiology:

- **Pharmaceutical**
  Production of:
  - antibiotics
  - hormones
  - vaccines
  - blood-clotting factors
- **Agricultural**
  - Manufacturing of pharmaceuticals for veterinary medicine
  - Plant genetic engineering
  - Cultivation of leguminous plants
- **Food**
  Production of:
  - dairy products
  - pickles
  - fructose and citric acid added to carbonated drinks
  - vitamins
  - wine, beer, spirits
- **Chemical**
  Manufacturing of:
  - alcohols
  - solvents
  - organic acids
- **Environmental protection**
  - biodegradation of pollutants
  - biosorption of pollutants

Figure 1 Industries that rely on microbiological processes.

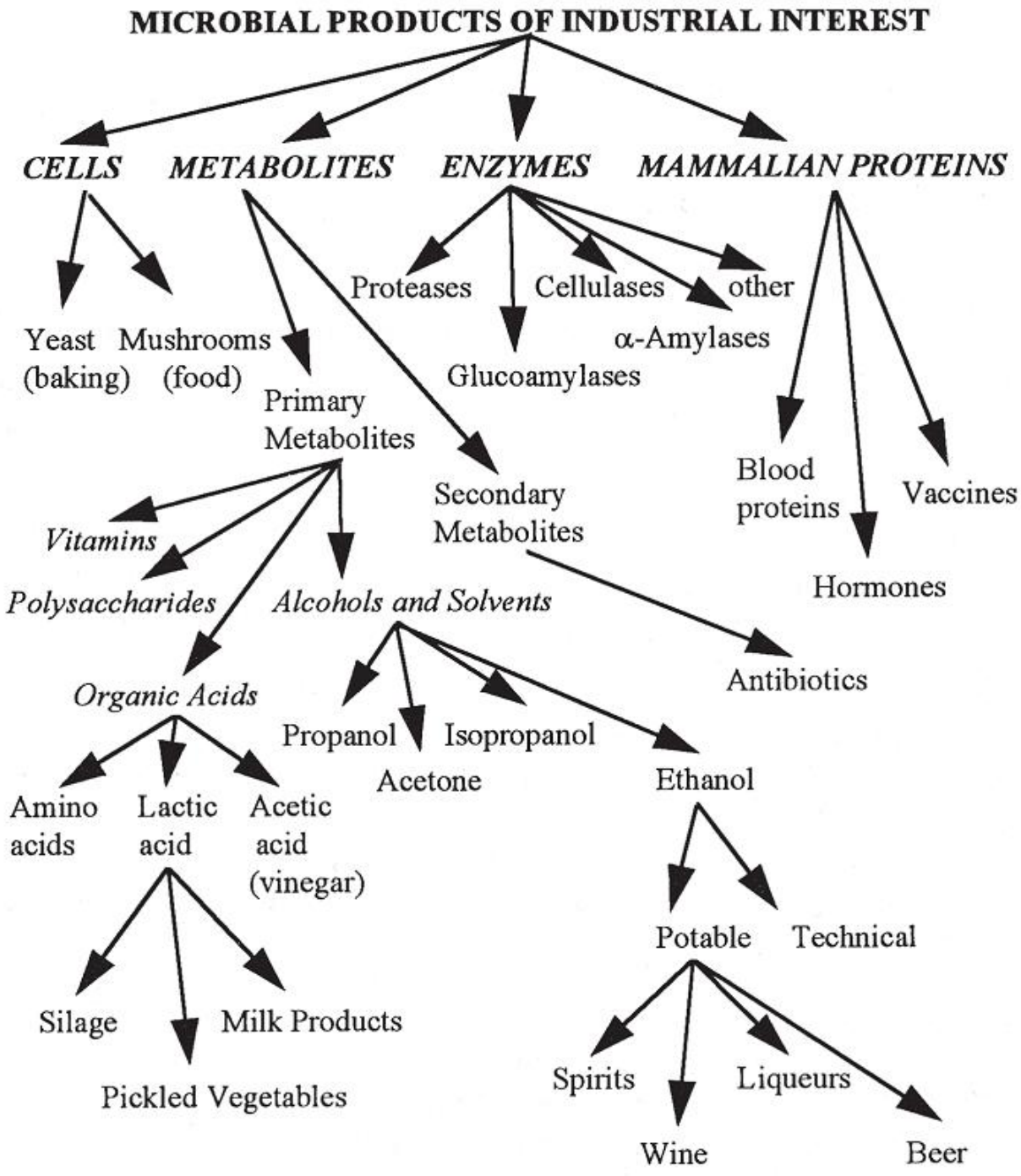

Figure 2 Important products of biotechnology

The objectives of this research were to investigate the bioactivity of I_E waters in aerobic and anaerobic bacteria and aerobic fungi (*in vivo* experiments) and to study the influence of I_E waters on activities of various bacterial and fungal enzymes (*in vitro* experiments).

2. Materials and Methods

2.1 Microbial Strains and Growth Conditions for In Vivo Experiments

Fungal strains FS11, FS22, FS33, and AA, and bacterial strains BL and PS were used for *in vivo* and *in vitro* experiments. All fungal and bacterial strains were initially grown on agar slants prepared with the appropriate growth media without I_E water added. Some amount of cells from the plates were suspended in a small amount of the growth medium with no I_E water to prepare an inoculum. The prepared inoculum was then added into the liquid production medium, prepared with different types of I_E waters or with distilled water as a control. The content of I_E waters in the cultivation media was about 100%.

The cultivations of FS11, FS22, and FS33 strains were carried out for 160-180 hr. in shaking glass flasks. At time intervals, samples were removed for assay of xylanase activity and CMCase activity, activity toward soluble carboxymethyl cellulose. All measurements were carried out in triplicate.

The BL, PS and AA strains were cultivated for 72-144 h. At different time intervals, samples were withdrawn for assay of α-amylase activity (BL and PS strains) and glucoamylase activity (AA strain). All measurements were repeated three times.

To demonstrate the in vivo bioactivity of I_E waters, agar nutrition medium was prepared with D_S-20 and D_S-94 I_E waters and with distilled water as a control. The prepared agar plates were seeded with AA strain from an agar slant (prepared with distilled water) and incubated at room temperature to obtain single colonies.

An effect of D_S-50 I_E solution on the activity of three anaerobic methanogenic consortia [from preacidification stage and methanogenic stage of pilot two-stage methanogenic reactor, and from industrial methanotank for utilization of municipal wastes] were studied. Experiments were carried out in lab-scale bioreactors (2.8 L plastic methanotanks), and were duplicated for each sample of methanogenic consortium.

The components of nutritional medium were dissolved either in distilled water (control) or in I_E D_S-50 water. Methanotanks were filled by 90% with the obtained media and inoculated with 10 % v/v of one of the three types of active biomass. The overall content of I_E D_S-50 water in methanotanks, therefore, was 90%. The cultivation was carried out in a batch-mode. Acetic acid was used as the substrate for methanogenic bacteria. It was added to the nutritional medium at the beginning of the cultivation, to give the initial concentration of 3 g/L.

CH_4 was measured in off-gas of the methanotanks by a gas chromatography (GC Type LHM 8 MD-3, Moscow Experimental Chromatography Plant, Russia) and the rate of CH_4 production was determined. The ratio of the methane production rate to dry weight of methanogenic consortium biomass was calculated. This ratio was used as a measure of the activity of methanogenic consortium.

2.2 Enzyme Preparations for In Vitro Experiments

Super ACE Blends (#1 and #2), crude liquid industrial preparations of cellulase and xylanase, were extracted from FS11 fungal strain. The crude dry preparation #3.29.1 of cellulase and xylanase was produced from FS22 fungal strain. The crude thy preparation F60-4 UF-FD #213.3 of cellulase and xylanase was prepared from the FS33 fungal strain. The crude industrial dry preparation AA G18x of glucoamylase was obtained from the AA fungal strain. The crude industrial dry preparations PS G3x of α-amylase and protease were produced from the bacterial PS strain. The crude industrial dry preparation BaL: 018x of α-amylase was made from the BL bacterial strain. The crude industrial dry preparation AA G10x of lipoxigenase was processed from the AA fungal strain.

2.3 Assays of Cellulase Activities

Cellulases are enzymes which split β-1,4-glucosidic bonds in cellulose. Most cellulases are made up of at least three different activities (several endo-β-1,4,- glucanases, cellobiohydrolases and a cellobiase). Another activity that is associated with cellulase and has an important role in hydrolytic breakdown of lignocellulosic biomass to single sugars is xylanase (breaks xylan).
The activities of cellulases towards soluble substrates were studied using IUPAC recommended standard carboxylmethylcellulase (CMCase), β-glucanase, β-glucosidase and xylanase activity assays as described elsewhere[4,5]. CMCase activity was measured as cellulase activity towards soluble carboxymethyl cellulose. β-glucanase activity was assayed as cellulase activity towards soluble barley β- glucan. Xylanase activity was measured as activity towards soluble birch wood xylan. Activity of β-glucosidase activity was measured as an initial rate of pnitrophenol production from soluble p-nitrophenyl-β-D-glucoside (pNPG). The activity of cellulase towards insoluble cellulose was analysed with filter paper assay (FPA). FPA reflects total saccharification cellulase activity in filter paper hydrolysis (initial rate of reducing sugar production).

The I_E waters were used to dilute cellulase preparations by 500-30,000 times and to prepare 0.1-0.2 M acetic, pH 5.0, buffer. The stock solutions of soluble substrates (CMC, (β-glucan, Xylan, pNPG) were prepared with distilled water. The insoluble filter paper substrate was suspended in I_E-based acetic buffer solution. The resulting content of I_E waters in filter paper assay was about 100% and it was 95% in β-glucosidase activity assay. Final I_E concentrations in CMCase, β-glucanase, and xylanase activity assays were about 50%. All experiments were carried out in triplicate in plastic vessels. Control assays were performed the same way, but with no I_E water in the reaction mixtures.

2.4 Assays of Glucoamylase, Lipoxigenase, α-Amylase, and Protease Activities

Glucoamylase is the exo-depolymerase, which hydrolyzes starch by non-random cleavage of α-1,4-glucosidic bonds from the terminal side of the polymeric molecule of starch. Glucoamylase activity was determined as an initial rate of glucose production from soluble corn starch at 30°C, pH 4.7.

Soluble starch was dissolved in I_E D_S-20 or D_S-94 water, boiled for 10 min., and then cooled to 20°C. The obtained solution was diluted with acetic buffer (pH 4.7), prepared with I_E D_S-20 or D_S-94, to give 1.5% w/v final concentration of starch. 10mL of this starch solution was mixed with 5mL of AsAw G18x α-amylase preparation, and incubated for 10 min. at 30°C. After the reaction was stopped, glucose concentration was determined.

α-amylase is the endo-depolymerase, which hydrolyzes starch by random cleavage of internal α-1,4-glucosidic bonds. α-amylase activity of PrSu G3x preparation was determined at pH 6.0 and 30°C and that of BaL G18x at pH 7.5 and 90°C as described below.

Soluble starch was dissolved in I_E D_S-20 or D_S-94 water, boiled for 10 min. and cooled to 20°C. The obtained solution was then diluted with phosphate buffer, to a pH of 6.0 or 7.5, prepared with I_E D_S-20 or D_S-94, to give 1.5% w/v final concentration of starch. 10mL of this starch solution was mixed with 5mL of PrSu G3x or BaL G18x α-amylase preparation, and incubated for 10 min. at the proper temperature. After the reaction was stopped, reducing sugars concentration was determined, and activity of the enzyme was calculated as an initial rate of reducing sugars production.

Protease is the enzyme, which catalyzes hydrolysis of peptide bonds in polypeptides and proteins. Protease activity toward casein was determined spectrophotometrically at 670nm.

Casein was dissolved in phosphate buffer (pH 8.5), prepared with I_E D_S-20 or D_S-94, to produce 2% w/v solution. 2mL of the obtained solution was mixed with PrSu G3x protease preparation and incubated for 15 min. at 55°C. The remaining (at the end of the reaction) substrate was precipitated with 4mL of 5% trichloroacetic acid (TCAA) and filtrated. A lmL sample was withdrawn from the filtered solution and mixed with 5mL of 0.5M Na_2CO_3 and lmL of Folin Reagent. The optical density was measured against an appropriate blank at 670nm.

Lipoxigenase (lipoxidase) activity was determined spectrophotometrically at 234nm. A mixture of linoleic, linolenic and arachidonic fatty acids was used as a substrate. Substrate stock solutions were prepared by adding 0.25 mL of the fatty acids mixture drop by drop to 5mL of universal buffer with a pH of 6 for fungal lipoxigenase or pH 9 for soybean lipoxidase, prepared in both cases with I_E water D_S-20 or D_S-94. The obtained solutions were homogenized by hand mixing and then they were diluted with the appropriate buffer to bring the volume to 50mL. Immediately before activity determination, the stock substrate solution was diluted 3.5 times with the buffer solution, with an appropriate pH. 10 mL of the diluted substrate solution was then mixed with lmL of AsAw G10x lipoxigenase preparation or soybean lipoxidase preparation (Sigma), and the mixture was incubated for 10 min. at 16°C. Optical density (234nm) was measured against appropriate blank at the end of the reaction.

The content of I_E waters in the reaction mixtures of glucoamylase, lipoxigenase, α-amylase and protease activity assays was about 100%. The same enzyme preparations, buffers and substrates were used for control assays, but without any I_E water added to the reaction mixture. All experiments were triplicated and they were carried out in plastic vessels. Magnetic stirring was not used to prevent any influence of magnetic field on charged I_E structures.

2.5 I_E Waters Used in the Study

Five types of I_E waters were used: D_S, A_{CE}, S_S, I_M, C_S. Four concentrations of D_S I_E water were studied: D_S-20, D_S-50, D_S-94, and D_S-310 (the larger the number in the code, the higher I_E concentration). Two concentrations of C_S (C_S-18 and C_S-80) and I_M (I_M-25 and I_M-70) I_E solutions were examined. Three concentrations of S_S (S_S-16, S_S-32, S_S-46) and one concentration of A_{CE} (A_{CE}-20) I_E solutions were used in the study.

All I_E water solutions were prepared by the same method, but using different substances (C_S-- cellulose; I_M -- isomaltose; S_S -- sophorose; A_{CE} -- cellulase; D_S -- proprietary solution) to initiate the process of I_E crystals' formation. First, very dilute solutions (10^{-13} M) of the above substances in ultra pure water (less than 10 ppb total dissolved solids, resistance of 18MΩ) were prepared by consequent dilution and mixing by shaking. The obtained solutions contained low levels of I_E crystals. They were further concentrated to produce I_E solutions of various concentrations [1,2].

3. Results and Discussion

3.1 In Vivo Experiments

3.1.1 Effect of I_E Waters on Fungal Strains, Producers of Cellulases and Xylanases

Fungal strains FS11, FS22 and FS33, producers of cellulases and xylanases, were used in this study. *In vivo* bioactivity of I_E waters was assessed by the influence of I_E waters on the level of maximum CMCase and xylanase activities observed in the course of fermentation in shaking flasks, and by the effect of I_E waters on the cultivation time required to attain the maximum CMCase or xylanase activities.

Table 1 illustrates *in vivo* bioactivity of various I_E waters in aerobic fungal strains FS11, FS22, and FS33 as compared to distilled water controls. A positive bioactivity toward FS11 strain was observed for all I_E waters tested except for C_S-18. The majority of I_E waters, with the exception for D_S-20, S_S-46, A_{CE}-20, and I_M-25, positively stimulated the FS33 strain. However, no effect or inhibiting activity of all I_E waters, aside from S_S-16 and A_{CE}-20, on FS22 strain, were observed.

3.1.2 Effect of I_E Waters on the Bacterial Strains BL and PS and the Fungal Strain AA

The effect of D_S type of I_E water at two concentrations (D_S-20 and D_S-94) on BL and PS bacterial strains, producers of α-amylase, and AA fungal strain, producer of glucoamylase, was investigated. The content of I_E waters in the fermentation media was about 100%. During the cultivation, samples of cultures were withdrawn and α-amylase activity (BL, PS strains) and glucoamylase activity (AA strain) were assayed. The results are shown in Table 2.

Table 1 Effect of I_E waters on the aerobic fungal strains, producers of cellulases and xylanases

Fungal strains	Type of I_E water	Effect of I_E waters on maximum enzymatic activity observed during fermentation		Effect of I_E waters on cultivation time required to attain maximum enzymatic activity	
		CMCase	Xylanase	CMCase	Xylanase
FS11	D_S-20	++	+	++	0
	D_S-50	++	++	++	0
	D_S-94	++	+	++	0
	D_S-310	+	+	0	+
	A_{CE}-20	+	-	-	0
	S_S-16	++	0	0	0
	S_S-32	+	+	0	0
	S_S-46	++	++	-	++
	I_M-25	-	++	+	++
	I_M-70	+	+	-	-
	C_S-18	0	-	0	0
	C_S-80	+	+	0	+
FS22	D_S-20	0	0	0	0
	D_S-50	0	0	0	0
	D_S-94	0	0	0	0
	D_S-310	0	0	0	0
	A_{CE}-20	+	+	0	0
	S_S-16	+	0	++	0
	S_S-46	0	0	0	0
	I_M-25	-	0	0	0
	I_M-70	+	0	0	-
FS33	D_S-20	0	0	0	0
	D_S-50	0	+	0	+
	D_S-94	++	+	+	+
	D_S-310	0	0	+	0
	A_{CE}-20	++	+	++	+
	S_S-16	+	+	0	0
	S_S-46	+	0	- -	-
	I_M-25	-	+	-	-
	I_M-70	++	++	+	0
	C_S-18	+	0	-	0
	C_S-80	-	++	+	++

(++) - increase of the parameter by more than 50% versus control
(+) - increase of the parameter by 5-50% versus control
(0) - insignificant, less than 5% change in the parameter as compared to control
(-) - decrease of the parameter by 5-25% compared to control
(- -) - decrease of the parameter by 25-50% versus control

Table 2 Effect of D_S-20 and D_S-94 I_E waters on a-amylase production by the BL and PS bacterial strains and glucoamylase production by the AA fungal strain.

Cultivation time, hr.	Change in enzymatic activity in samples prepared with D_S -20 versus controls , %			Change in enzymatic activity in samples prepared with D_S -94 versus controls , %		
	BL strain (α-amylase)	PS strain (α-amylase)	AA strain (gluco-amylase)	BL strain (α-amylase)	PS strain (α-amylase)	AA strain (gluco-amylase)
72	10	-6	ND	3	-10	ND
96	42	-9	ND	4	6	ND
120	51	-21	ND	-3	-17	ND
168	ND	ND	-11	ND	ND	-25

ND - parameter was not determined.
The positive numbers indicate increase and the negative numbers show decrease in the enzymatic activity.

Ds-20 I_E water stimulated a significant increase of α-amylase activity in the BL strain (up to 50% versus control), but it slightly inhibited α-amylase production in the PS strain (up to 20%) and glucoamylase production in the AA fungal strain (up to 10%). D_S-94 I_E water was neutral or inhibitory for all strains tested.

3.1.3 Effect of Diluted (D_S-20) and Concentrated (D_S-94) I_E Waters on Morphology and Density of Fungal Colonies Grown on Agar Plates.

Agar solid growth media were prepared with D_S-20, D_S-94 and distilled water as a control. The prepared agar plates were seeded with the AA fungal strain and incubated at room temperature.

Apparent morphological differences between colonies grown on agar plates with D_S I_E waters and distilled water were observed. The addition of D_S-20 I_E water to the growth media produced more colored pigment colonies with more spores. On the contrary, colonies detected on agar plate prepared with D_S-94 were transparent, without any pigment, and with very low sporulation.

3.1.4 Effect of D_S-50 I_E Water on Consortia of Anaerobic Methanogenic Microorganisms

Consortium of anaerobic microorganisms is widely used in large scale for digestion and utilization of organic wastes and for production of "bio-gas" (70% of CH_4 and 30% of CO_2 mixture). The effects of D_S-50 I_E water on three methanogenic consortia were studied: a consortium from the preacidification stage, of pilot two-stage methanogenic reactor; a consortium from the methanogenic stage of the same reactor, and a consortium from industrial methanotank for utilization of municipal wastes.

The addition of D_S-50 to the growth medium of the consortium from the methanogenic stage of pilot scale reactor led to a significant increase (1.7 times over control) of methane production. The other two consortia were not stimulated by D_S-50. Although a further detailed study is required, a conclusion can be made about the possibility of using I_E in an industrial setting for boosting the efficiency of municipal waste digestion.

3.2 In Vitro Experiments

3.2.1 Effect of I_E Waters on Cellulase and Xylanase Activities

The effects of various types and concentrations of I_E waters on the activity of fungal cellulases and xylanases were studied. Since cellulases are made up of at least three different activities, assays for carboxylmethylcellulase (CMCase), β-glucanase, and β-glucosidase activities were carried out. Overall activity of cellulases towards insoluble cellulose was analysed with filter paper assay (FPA). The obtained results are shown in Table 3.

Based on the analysis of Table 3, a conclusion can be drawn about a prominent stimulating effect of the tested D_S, A_{CE}-20, S_S-46, and C_S-18 I_E waters on cellulase and xylanase activities in the FS11, FS22, and FS33 fungal strains. On the other hand, S_S-16, S_S-32, I_M-25, I_M-70, and C_S-80 I_E water preparations have a neutral or slightly inhibiting effect on cellulase and xylanase activities in the studied fungal strains.

3.2.2 Effect of I_E Waters on Glucoamylase, Lipoxigenase, α-Amylase, and Protease Activities

The effects of various types and concentrations of I_E waters on the activity of fungal glucoamylase and lipoxigenase, and bacterial α-amylase and protease were studied

Table 3 Effect of I_E waters on cellulase and xylanase activities

Fungal enzyme blends	Type of I_E water	Enzymatic activities assayed				
		FPA (overall cellulase)	CMCase	β-Glucanase	Xylanase	β-Glucosidase
Super Ace Blend #2, FS11 strain	D_S-20	+	+	+	+	++
	D_S-50	+	+	+	++	++
	D_S-94	+	+	+	++	ND
	D_S-310	+	+	++	+++	ND
	A_{CE}-20	+	++	++	+	ND
	S_S-16	0	-	-	-	-
	S_S-32	+	-	-	+	0
	S_S-46	+	+	++	+	++
	I_M-25	0	-	-	-	0
	I_M-70	+	-	-	0	0
	C_S-18	+	+	++	+	+
	C_S-80	+	-	-	+	++
Super Ace Blend #1, FS11 strain	D_S-20	+	++	+	+	+
	D_S-50	+	++	+	+	+
	D_S-94	+	+++	+	++	0
Blend # 3.29.1, FS22 strain	D_S-20	+	+	++	+	+
	D_S-50	+	+	++	++	+
	D_S-94	+	+	+	++	0
Blend #213.1, FS33 strain	D_S-20	0	++	+++	+	0
	D_S-50	0	+	++	+	+
	D_S-94	0	+	++	+	-

(+++) - over 50% enzymatic activity increase over control
(++) - 25 to 50% enzymatic activity increase over control
(+) - 5 to 25% enzymatic activity increase over control
(0) - insignificant, less than 5% change in the activity as compared to control
(-) - 5 to 25% activity decrease compare to control
(ND) - activity was not determined

Table 4 Effect of I_E waters on glucoamylase, lipoxigenase, α-amylase, and protease activities

Type of I_E water	Enzymatic activities assayed			
	Glucoamylase (fungal preparation AA G18x)	Lipoxigenase (fungal preparation AA G18x)	α-Amylase (bacterial preparation PS G3x)	Protease (bacterial preparation PS G3x)
D_S-20	0	+	0	-
D_S-50	ND	ND	ND	ND
D_S-94	0	+++	0	+
D_S-310	-	ND	-	+
A_{CE}-20	-	ND	-	+
S_S-16	0	ND	-	+
S_S-32	0	ND	-	+
S_S-46	0	ND	0	0
I_M-25	-	ND	-	-
I_M-70	0	ND	-	0
C_S-18	0	ND	0	+
C_S-80	0	ND	0	0

(+++) - over 50% enzymatic activity increase over control
(++) - 25 to 50% enzymatic activity increase over control
(+) - 5 to 25% enzymatic activity increase over control
(0) - insignificant, less than 5% change in the activity as compared to control
(-) - 5 to 25% activity decrease compare to control
(ND) - activity was not determined

in vitro. The content of I_E waters in the reaction mixtures was about 100%. The obtained results are shown in Table 4.

Data presented in Table 4 demonstrate neutral or slightly negative effect of tested I_E waters on glucoamylase and α-amylase activities. A slight stimulation of protease activity (up to 25% over control) was observed with D_S-94, D_S-310, A_{CE}-20, S_S-16, S_S-32, and C_S-18 I_E preparations, while the other I_E waters were slightly inhibiting. Fungal lipoxigenase was strongly stimulated (up to 120% over control) with D_S-94 I_E sample, and only slightly (about 10% increase over control) with D_S-20.

The *in vivo* and *in vitro* data presented above strongly suggest a possibility for development of customised I_E waters for each particular microorganism and enzyme. Such I_E preparations can be used in enzyme manufacturing processes instead of water to increase yields and activities of the produced enzymes. Similarly, a customized I_E preparation can be mixed with a purified enzyme to maximize its activity and to reduce its consumption in an application.

4. References

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